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13. ABSTRACT (Maximum 200 Words)

The project evaluated anticonvulsant and neuroprotectant properties of novel medical countermeasures to lithium-pilocarpine-induced status epilepticus (SE)used here as a model of organophosphorus nerve agents. Although mefenamate induced modest anticonvulsant effects, survival was reduced and the SE-induced neuropathology was exacerbated. Nicotinamide induced only modest anticonvulsant and neuroprotectant activity at the doses tested. N-acetylcysteine was not anticonvulsant but enhanced both neurological deficit and neuropathology. PBN, but not S-PBN, induced significant neuroprotection. ACPC induced neuroprotection in temporal regions without anticonvulsant activity. D-cycloserine had no effect. Propofol induced significant neuroprotection and anticonvulsant activity in the rat model tested. It is recommended that PBN and ACPC be tested in multiple dose regimens for long term neuroprotetion in nerve agent SE.

14. SUBJECT TERMS
S-PBN, PBN, nicotinamide, mefenamate, N-acetylcysteine

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INTRODUCTION

This project tested novel treatment strategies for the N-methyl-D-aspartic acid (NMDA) component of organophosphorous (OP) nerve agents that are intended to complement or improve current treatments. OP agents induce an initial cholinergic receptor over stimulation that is followed by a glutamatergic over stimulation of NMDA receptors. The excessive NMDA activation and excitotoxicity results in the status epilepticus (SE), neurological deficit and neuropathology associated with OP intoxication. The lithium-pilocarpine (Li-pilo) model of cholinergic convulsions in rats is used as the experimental model of OP nerve agent-induced SE. The project tests neuroprotectants that protect the brain from seizure-induced neuropathology by interfering with NMDA receptor-mediated intracellular excitotoxicity mechanisms. Experimental parameters used in these studies to assess the test drug activity were: 1) electrocorticograph (ECoG) determination of continuous high amplitude spiking as a measure of SE duration; 2) spontaneous activity before and after SE as a measure of neurological deficit; 3) neuropathology in brain regions damaged by Li-pilo SE.

The candidate medical countermeasures tested in the last 12 months of the project were mefenamate, nicotinamide and N-acetylcysteine. The results of those studies are presented in detail. The results of the α-phenyl-N-*tert*-butylnitrone (PBN) study are provided in the Appendix in manuscript form (Appendix Item 1). The results of the PBN study are also discussed in this final report. The final outcome of the1-aminocyclopropanecarboxylic acid (ACPC) and D-cycloserine (DCS) study are also discussed briefly with appropriate reference to the NeuroToxicology reprint provided in the Appendix (Appendix Item 2).

BODY

Tasks Completed from the Approved Statement of Work

The original Statement of Work was to study the anticonvulsant and neuroprotectant effects of mefenamate, nicotinamide, N-acetylcysteine, a-phenyl-N-*tert*-butyl nitrone (PBN), 1-aminocyclopropanecarboxylic acid (ACPC) and D-cycloserine (DCS) in the lithium-pilocarpine model of organophosphorus status epilepticus. All tasks were completed and are descried below.

The project objective over the last 12 months was to use the Li-pilo model of SE in rats to test the anticonvulsant and neuroprotectant activity of mefenamate, nicotinamide and N-acetylcysteine against the NMDA component of cholinergic convulsions. Li administration (3 mmol/kg, SQ) followed 20-24 hours later by pilocarpine (25 mg/kg, SQ) induces a SE of 2-3 hours duration that serves as a model of the convulsions induced by OP nerve agents. SE-induced afterdischarge was defined as the duration of continuous high amplitude ECoG spiking. Propofol (55 mg/kg, i.p.) was administered 3 hours following SE onset. As reported in the first progress report propofol significantly enhanced 24 hour survival without affecting the neuropathology induced by 3 hours of SE. The test compounds were to be administered either immediately following (within 1 minute) the pilocarpine administration (exposure treatment) or 5 minutes following the onset of SE (5 min SE treatment).

Mefenamate

Mefenamic acid (mefenamate) is a nonsteroidal anti-inflammatory drug (NSAID) that induces anticonvulsant activity in experimental models of epilepsy. Forty mg/kg mefenamic acid completely inhibits the seizures and neural damage induced by a convulsive dose (380 mg/kg) of pilocarpine (Ikonomidou-Turski et al., 1988). Mefenamate is also anticonvulsant in pentylenetetrazol-induced seizures in rats (Wallenstein and Mauss, 1984; Wallenstein, 1991) as well as seizures induced by systemic penicillin administration in rats (Wallenstein, 1987). However, the anticonvulsant activity is dose related as higher doses of mefenamate induce proconvulsant activity. For example, 60 mg/kg mefenamate significantly increased the number of seizures induced in a rat pentylenetetrazol kindling paradigm (Wallenstein, 1991). In addition, 50 mg/kg mefenamate reduced the latency to pentylenetetrazol seizure onset and enhanced the electrographic seizure activity induced by pentylenetetrazol (Wallenstein, 1985a). Of particular relevance to this report, 50 and 150 mg/kg mefenamate by itself induced high voltage, high frequency ECoG spiking when administered to rats (Wallenstein, 1985b).

Mefenamate was shown to be neuroprotective in a retina ischemic injury model (Chen et al., 1998). This effect was proposed to result from mefenamate inhibition of NMDA-evoked currents (Chen et al., 1998). Fenamate is also reported to inhibit nonselective cation channels which may result in neuroprotective effects (Partridge and Valenzuela, 2000). Neuroprotection may also result from mefenamate directly scavenging nitric oxide (NO) as has been demonstrated in vitro (Asanuma et al., 2001). While mefenamate is a cyclooxygenase inhibitor, this is less likely to be the mechanism of neuroprotection as the inhibition of both the cyclooxygenase and lipoxygenase pathways are necessary to

reduce SE-induced neuropathology in kainic acid seizures (Baran et al., 1994; Kim et al., 2000).

The mefenamate study produced interesting results. The 40 mg/kg mefenamate dose administered as exposure treatment produced anticonvulsant effects as 3 of the 9 rats failed to develop SE. In contrast, the rats in the same treatment group that did develop SE exhibited significantly greater neuropathology in thalamic and hippocampal regions than control rats. In contrast, 4 of the 8 rats treated with 40 mg/kg mefenamate following 5 minutes of Li-pilo SE (SE treatment) did not survive the 24 hour period prior to sacrifice. However, the rats that survived exhibited significantly reduced neuropathology in the amygdala.

Of further interest, 40 mg/kg mefenamate actually induced seizure activity. Within 5 minutes of i.p. administration limbic seizure activity was induced (Figure 1). These limbic seizures consisted of staring, chewing, nodding and forelimb clonus that were consistent with a kindled seizure rank of 3 as defined by Racine (1972). Several of these isolated rank 3 limbic seizures typically occurred in each animal. This was followed by a period of immobility with a loss of righting reflex. During this period of immobility the ECoG activity consisted of high frequency, high voltage spiking similar to that described by Wallenstein (1985). Following the 10-15 minute period of nonconvulsive seizure activity the animals regained mobility and resumed exploration of the cage. Three of the 9 rats administered 40 mg/kg mefenamic acid exposure treatment did not develop SE. The behavioral and neuropathology data of the 3 rats that did not experience SE were evaluated separately from the 6 rats that did experience SE.

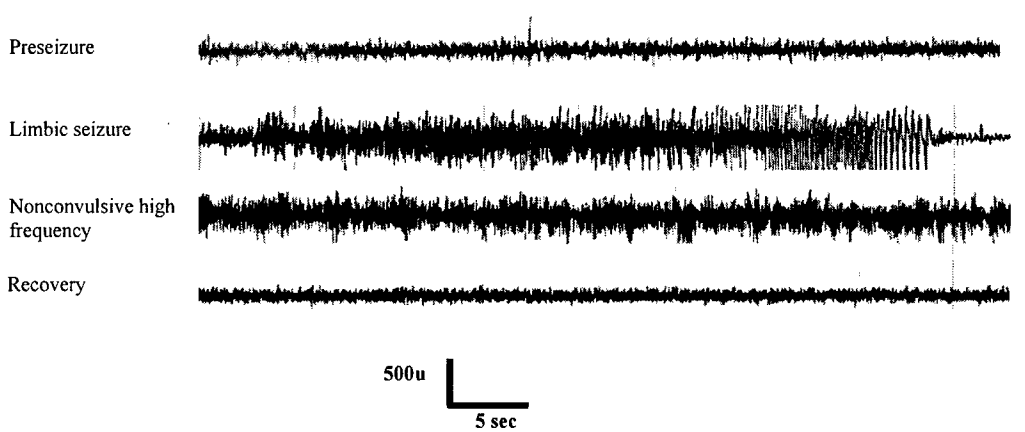


Figure 1. ECoG seizure afterdischarge activity induced by mefenamate. Initial epileptiform activity consisted of limbic seizures that were associate with isolated ECoG spiking activity similar to that induced by limbic kindling. This was followed by a period of continuous high frequency, high amplitude spiking associated with a period of immobility and loss of righting reflex.

Forty mg/kg mefenamate administered 5 minutes following SE onset reduced survival. Four of the 8 rats tested did not survive the 24 hour period following the mefenamic acid administration. The behavioral and neuropathology data from those animals is not included in the analysis.

Mefenamate exhibited anticonvulsant activity when administered as exposure treatment. Forty mg/kg administered as exposure treatment significantly increased the latency to SE onset (Figure 2). As noted previously, 3 of 9 rats administered 40 mg/kg mefenamate as exposure treatment did not develop Li-pilo SE. In addition, both 20 and 40 mg/kg mefenamate (exposure treatment) significantly reduced the Li-pilo SE afterdischarge duration as defined by continuous high amplitude ECoG spiking (Figure 3).

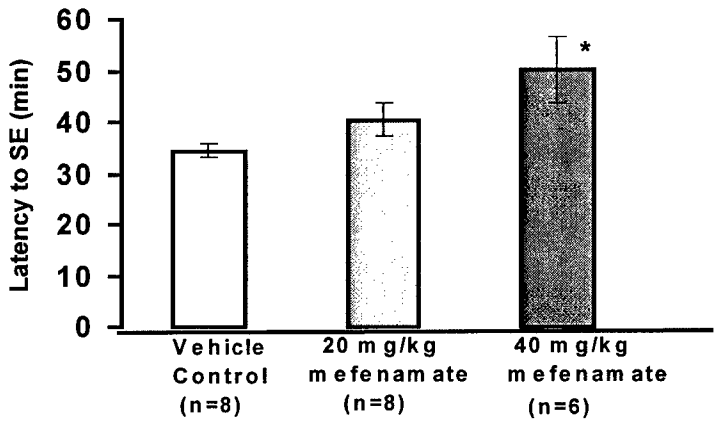


Figure 2. Latency to SE onset was the time from pilocarpine administration to the start of SE as defined by continuous high amplitude ECoG spiking. 40 mg/kg mefenamate administered as exposure treatment significantly delayed the onset of SE (one way ANOVA). Three additional rats did not develop SE.

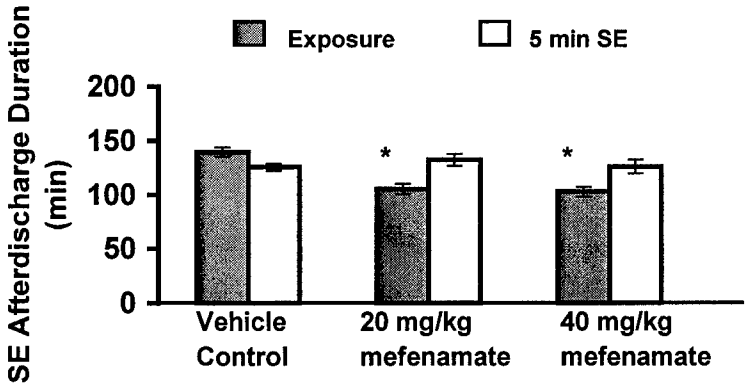


Figure 3. SE afterdischarge duration was defined as the period of continuous high amplitude ECoG spiking. Administration of 20 or 40 mg/kg mefenamate as exposure treatment significantly reduced the duration of SE (one way ANOVA). Data includes only those animals that exhibited SE.

Administration of mefenamate as exposure treatment has no effect on distance traveled (DT) or resting time (RT) as determined in the spontaneous activity assessment (Figure 4).

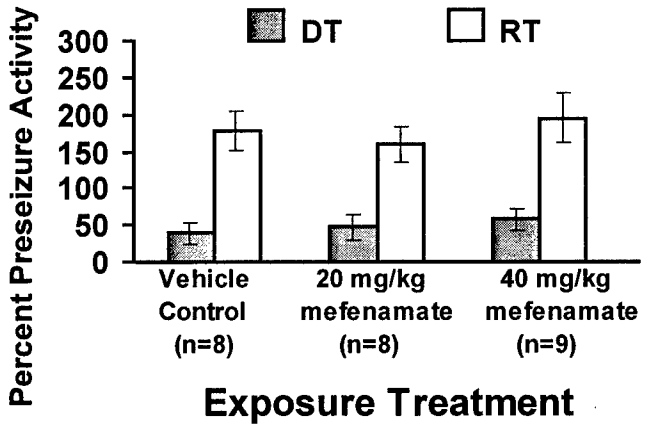


Figure 4. Effects of Li-pilo SE on spontaneous activity expressed as a percent of preseizure activity. Spontaneous activity was determined for 10 minutes in an activity monitor. Distance traveled (DT) was decreased and resting time (RT) increased indicating a SE-induced decrease in spontaneous activity. Mefenamate exposure treatment had no effect on the neurological deficit.

Likewise, administration of mefenamate following 5 minutes of Li-pilo SE had no effect on DT or RT (Figure 5).

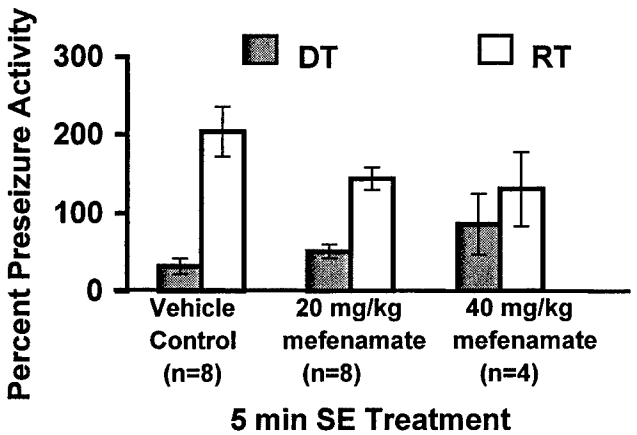


Figure 5. Mefenamate administration following 5 minutes of Li-pilo-induced SE had no effect on neurological deficit as measured by distance traveled (DT) and resting time (RT).

The spontaneous activity of the rats protected from SE did not differ significantly from that of vehicle control animals experiencing SE. The 3 rats treated with 40 mg/kg mefenamate as exposure treatment that did not develop SE exhibited average DT and RT values that did not differ significantly from control (Figure 6). It should be noted that the average distance traveled (DT) in this group of rats without SE was 100% of the preseizure control value. Although surprising considering that the animals experienced less than 10 minutes of SE, failure to show significance with the t-test is likely due to the variability among the small number (N=3) of animals protected from SE.

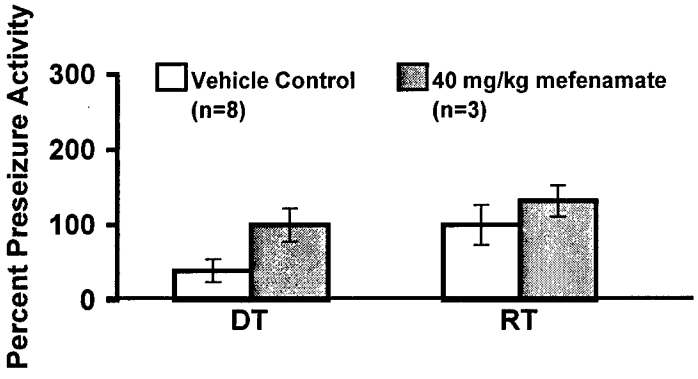


Figure 6. Average spontaneous activity of the 3 rats in which 40 mg/kg mefenamic acid inhibited Li-pilo-induced SE. The average percent preseizure activity did not differ significantly from the vehicle control group.

Twenty-four hours following pilocarpine administration and immediately following spontaneous activity assessment the animals were sacrificed and perfusion fixed for histological analysis of SE-induced neuronal damage. The 24 hour survival period is optimal for the demonstration of neuopathological damage by histopathology in both Li-pilo (Clifford et al., 1987; Fujikawa, 1996) and soman SE (McDonough et al., 1998). Formalin-fixed brains were removed and embedded in paraffin for sectioning. A 5 micron section was taken every 150 microns through the brain tissue 0.8 to 4.8 mm posterior to bregma. This area was chosen for the histopathological analysis for efficiency and because it contains critical brain nuclei that exhibit the greatest degree of damage from soman (McDonough et al., 1998) and Li-pilo convulsions (Clifford et al., 1987; Motte et al., 1998; Fujikawa et al., 1999; Peredery et al., 2000). The sections were prepared with hematoxylin and eosin (H&E) staining and sent to the coinvestigator, Dr. James Griffith of the Hershey Medical Center-Penn State University, for pathological analysis.

Table 1. Mean neural damage scores following mefenamate treatment for brain structures in the region –0.8 to –4.8 mm from bregma. * indicates significant differences from vehicle control group as determined by Kruskal-Wallis H-test. The underlined values indicate significant reductions in neuropathology.

	Exposure treatment			
Brain region	control n=8	20 mg/kg n=8	40 mg/kg (SE) n=6	40 mg/kg (no SE) n=3
parietal cortex (Par1)	2.65	3.26	2.88	0.98*
occipital cortex (Oc)	2.69	2.92	2.67	0.73*
perirhinal cortex (PRh)	3.63	3.41	2.54*	0.78*
piriform cortex (Pir)	4.00	4.00	3.80	0.91*
cortical amygdala (Co)	3.93	4.00	3.76	1.00*
medial amygdala (BM)	3.13	3.73*	3.37	1.05*
lateral amygdala (La)	3.58	3.88	3.40	0.69*
mediodorsal thalamus (MD)	2.14	4.00*	3.47*	2.24
lateraldorsal thalamus (LD)	2.11	4.00*	3.43*	1.90
pretectal nucleus (APTD)	2.06	3.73*	2.86*	1.96
CA3	2.37	3.83*	3.34*	1.21*
CA1	1.25	2.63*	2.16*	1.07
amygdalopiriform (Apir)	4.00	4.00	4.00	0.72*

	5 minute SE		
Brain Region	control n=8	20 mg/kg n=8	40 mg/kg n=4
parietal cortex (Par1)	3.03	3.07	2.73
occipital cortex (Oc)	2.88	3.36	2.77
perirhinal cortex (PRh)	3.71	3.64	2.88*
piriform cortex (Pir)	3.97	4.00	3.35
cortical amygdala (Co)	4.00	4.00	3.61*
medial amygdala (BM)	3.56	3.61	2.98*
lateral amygdala (La)	3.88	3.96	3.27*
mediodorsal thalamus (MD)	3.11	3.90*	3.48*
lateraldorsal thalamus (LD)	3.25	3.81*	3.61
pretectal nucleus (APTD)	2.90	3.61*	2.81
CA3	2.90	3.56*	3.50*
CA1	1.52	1.83	3.28*
amygdalopiriform (Apir)	4.00	4.00	3.72*

Three of 9 animals administered 40 mg/kg mefenamate as exposure treatment did not develop SE. As might be expected these animals had significantly reduced neuropathology scores in the cortical and amygdala regions (Table 1). In contrast, rats administered either 20 or 40 mg/kg mefenamate (exposure) that developed SE had significantly increased neuropathology in the thalamic and hippocampal regions (Table 1).

Four of 8 rats administered 40 mg/kg mefenamate 5 minutes following SE onset did not survive. However, the 4 that survived exhibited significantly reduced neuropathology in the perirhinal cortex and amygdala regions (Table 1). In contrast, 20 mg/kg mefenamate (5 min SE) significantly enhanced neuronal damage in the thalamus and hippocampus.

Mefenamate induced a modest anticonvulsant effect. Exposure treatment delayed onset of SE and reduced SE duration. Exposure treatment with 40 mg/kg mefenamate prevented SE in 3 of 9 rats. However, this minor anticonvulsant activity was associated with serious adverse effects. Half the rats (4 of 8) treated with 40 mg/kg mefenamate following 5 minutes SE did not survive overnight. Rats experiencing SE when treated with mefenamate exhibited significantly enhanced neuropathology in amygdala and hippocampus regions. In addition, the 40 mg/kg dose induced seizure activity by itself. Taken together this data would indicate that the adverse effects outweigh the potential therapeutic effect and that mefenamate should not be developed further as a candidate medical countermeasure for OP nerve agent SE.

The anticonvulsant activity of mefenamate in Li-pilo SE was markedly different from that reported by Ikonomidou-Turski et al. (1988) in pilocarpine seizures. In that study 40 mg/kg mefenamate administered 45 minutes prior to pilocarpine (380 mg/kg) totally suppressed seizure development. In the present study Li-pilo seizures were inhibited in only 3 of 9 rats when administered immediately following pilocarpine (exposure treatment). The same dose administered during SE (SE treatment) did not affect seizures and actually reduced survival. Methodological differences may account for the discrepancy including rat strain (Wistar vs. Sprague-Dawley in the present study), seizure model (pilocarpine alone vs. Li-pilo) and time of mefenamate administration. Of particular concern was the proconvulsant activity induced by mefenamate itself. The seizure threshold reducing effect of mefenamate has also been observed in other laboratories (Wallenstein, 1985; Kelly and Auer, 2003). The introduction of a proconvulsant drug in a population of OP nerve agent exposed individuals at risk of SE would be problematic.

Although initially reported to be neuroprotective in a retina ischemia model (Chen et al., 1998), mefenamate induced no neuroprotection in experimental cerebral ischemia (Kelly and Auer, 2003). Our results were mixed with both neuroprotection and an exacerbation of neuropathology being observed following mefenamate administration. Mefenamate enhanced the neuropathology observed in the thalamus and hippocampus. Neuroprotection was observed in the perirhinal cortex and amygdala but this was in the 40 mg/kg 5 minutes SE treatment group with four rats (N=4). It should be noted that the

number of rats in this group was reduced by poor survival and the data may not be statistically reliable. We conclude that mefenamate neuroprotection was negligible in this model of OP nerve agent exposure.

Mefenamate posses numerous pharmacological properties. In addition to being a cyclooxygenase inhibitor it inhibits nonselective cation channels (Partridge and Vanenzuela, 2000), scavenges nitric oxide (Asanuma et al., 2001) and inhibits NMDA mediated currents (Chen et al., 1998). Any of these mechanisms might be a means to induce neuroprotective or anticonvulsant effects. It is also conceivable that this set of nonselective effects contributed to the lowered seizure threshold and reduced survival observed following mefenamate.

Nicotinamide

Nicotinamide is a poly(ADP-ribose) polymerase (PARP) inhibitor. NMDA receptor activation initiates nitric oxide (NO) production. NO reacts with superoxide to produce peroxynitrite and hydroxyl radical (Elliasson et al. 1997). The radicals damage DNA thus activating PARP (Eliasson et al. 1997; Pieper et al. 1999). PARP catalyzes the addition of long branched chains of poly(ADP-ribose) from the substrate nicotinamide adenine dinucleotide (NAD^+) to numerous nucleus proteins including DNA and PARP itself (Eliasson et al. 1997; Pieper et al. 1999). This is hypothesized to act as a protective repair mechanism for the DNA damage. However, massive DNA damage such as that induced in cholinergic convulsions, elicits excessive PARP activation that ultimately depletes NAD^+ and ATP leading to cell death.

PARP inhibitors are neuroprotective. The PARP inhibitors benzamide and 3-amino benzamide block NMDA and NO-mediated neurotoxicity in rat cerebral cortex cultures (Zhang et al., 1994) and cultured cerebellum granule cells (Cosi et al., 1994). These inhibitors also protect against ischemia-induced damage in rat brain (Lo et al., 1998) and attenuate methamphetamine-induced dopamine depletion (Cosi et al., 1996). Most significantly, benzamide induces significant reductions in soman SE-induced lesion size in rat piriform cortex (Meier et al., 1999).

Nicotinamide is the PARP inhibitor used in this study because it is readily available, is widely administered to humans and is known to have low toxicity in humans. Besides inhibiting PARP, nicotinamide is a free radical scavenger and is also a precursor of NAD^+, which would help correct the energy depletion, resulting from DNA damage-induced PARP activation (Pieper et al., 1999; Gale 1996a; 1996b). Nicotinamide protects against NO-induced damage in the rat hippocampus slice (Wallis et al., 1993). Nicotinamide produces significant reduction in lesion size following focal cerebral ischemia in rats even when administered up to 2 hours following the ischemic insult (Ayoub et al., 1999; Sakakibara et al., 2000; Toshihiko et al., 2000). In addition, the 2 hour delayed nicotinamide treatment improves neurological and sensory function as well as reduces lesion volume when tested up to 7 days following permanent middle cerebral artery occlusion (Toshihiko et al. 2000). This suggests nicotinamide induces long-term neuroprotective effects of brain function that may be of significant value as a medical countermeasure to OP nerve agents.

Nicotinamide administered as exposure treatment induced a modest anticonvulsant effect. One of 8 rats in both the 150 and 300 mg/kg dose groups failed to develop SE. In the animals that did develop SE, nicotinamide increased the latency to SE onset. Both 150 and 300 mg/kg nicotinamide (ip) administered immediately following pilocarpine delayed the onset of the cholinergic convulsions (Figure 7).

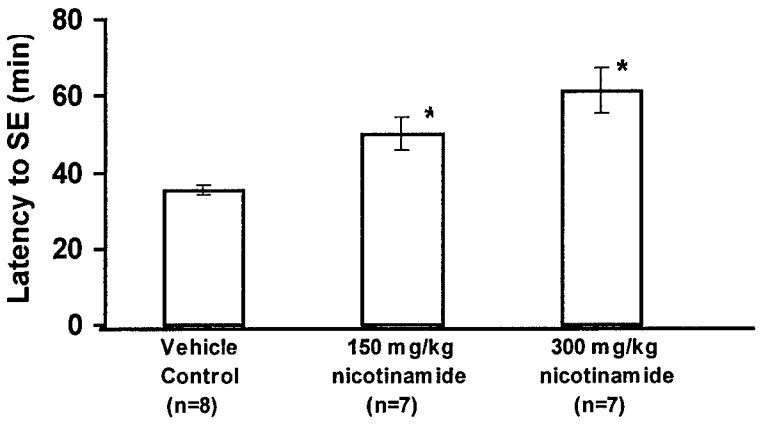


Figure 7. Latency to SE onset was the time from pilocarpine administration to the start of SE as defined by continuous high amplitude ECoG spiking. 150 and 300 mg/kg nicotinamide significantly delayed the onset of SE (one way ANOVA). One of the 8 animals tested in each of the nicotinamide dose groups failed to develop SE.

Nicotinamide had little effect on ECoG afterdischarge duration. 150 mg/kg nicotinamide administered as exposure treatment induced a small but significant reduction in afterdischarge duration (Figure 8). No other dose or administration point induced any significant change in SE afterdischarge duration.

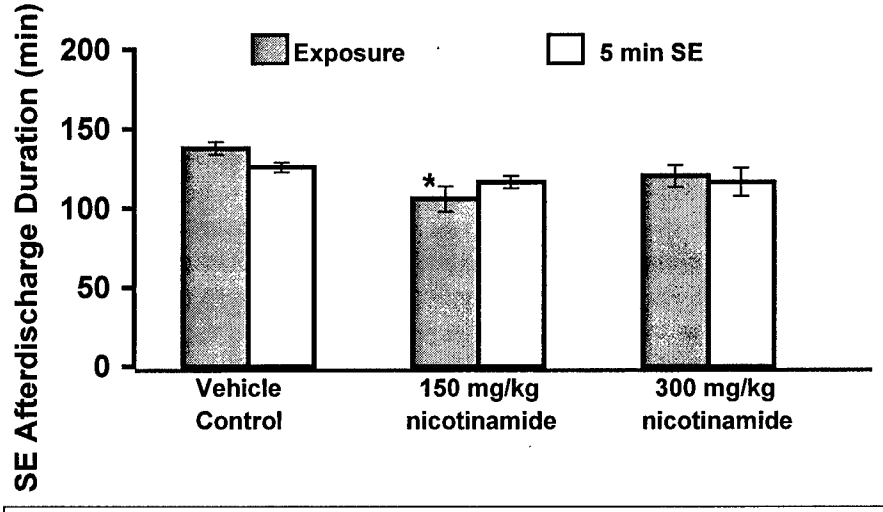


Figure 8. SE afterdischarge duration defined as the period of continuous high amplitude ECoG spiking. Only the 150 mg/kg nicotinamide exposure treatment induced a significant decrease in afterdischarge duration (one way ANOVA). Data includes all animals tested.

Administration of nicotinamide as exposure treatment had no effect on distance traveled (DT) or resting time (RT) as determined in the spontaneous activity assessment (Figure 9).

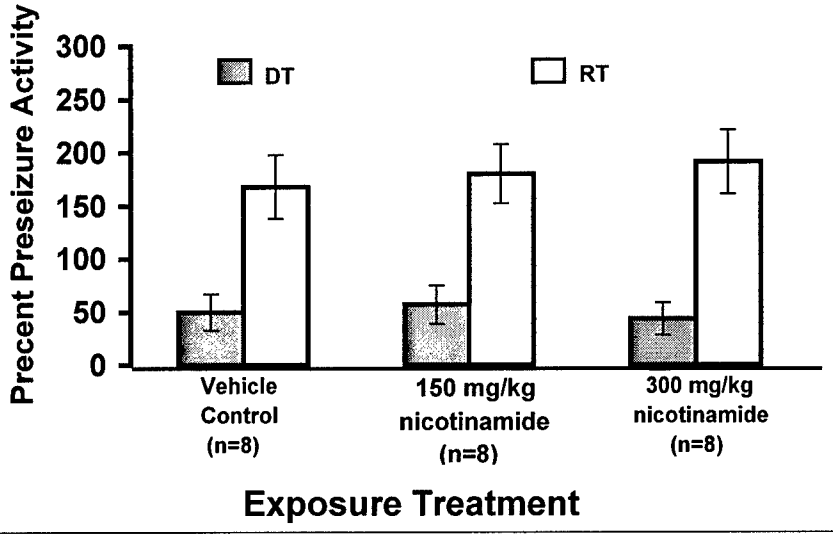


Figure 9. Effects of Li-pilo SE on spontaneous activity expressed as a percent of preseizure activity. Spontaneous activity was determined for 10 minutes in an activity monitor. Distance traveled (DT) was decreased and resting time (RT) increased indicating a SE-induced decrease in spontaneous activity. Nicotinamide exposure treatment had no effect on the neurological deficit.

Likewise, administration of nicotinamide following 5 minutes of Li-pilo SE had no effect on DT or RT (Figure 10).

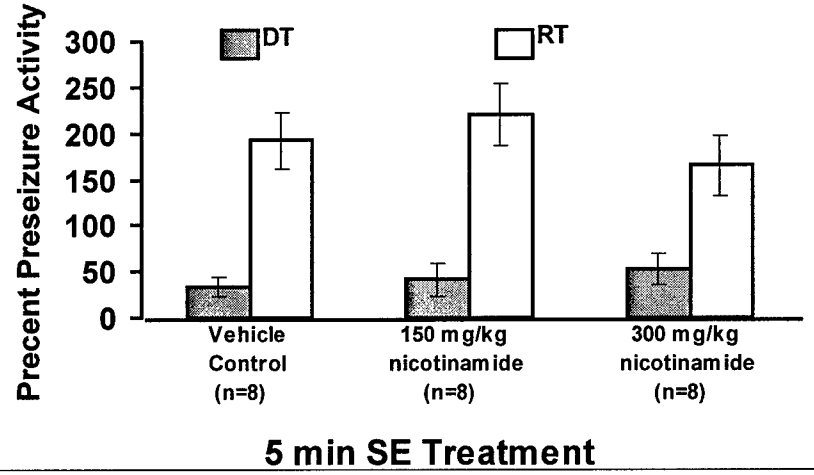


Figure 10. Nicotinamide administration following 5 minutes of Li-pilo-induced SE had no effect on neurological deficit as measured by distance traveled (DT) and resting time (RT).

Twenty-four hours following pilocarpine the animals were sacrificed and perfusion fixed for histological analysis of SE-induced neuronal damage. The results of the analysis are presented in Table 2.

Table 2. Mean neural damage scores following nicotinamide treatment for brain structures in the region –0.8 to –4.8 mm from bregma. * indicates significant differences from vehicle control group as determined by Kruskal-Wallis H-test. The underlined values indicate significant reductions in neuropathology.

	Exposure treatment		
Brain region	**control n=8**	**150 mg/kg n=8**	**300 mg/kg n=8**
parietal cortex (Par1)	2.58	3.02	1.58*
occipital cortex (Oc)	2.69	2.78	2.08
perirhinal cortex (PRh)	3.54	3.15	2.16*
piriform cortex (Pir)	4.00	3.50	2.96*
cortical amygdala (Co)	3.91	3.50	3.07
medial amygdala (BM)	3.09	3.20	3.16
lateral amygdala (La)	3.46	3.50	3.23
mediodorsal thalamus (MD)	2.01	3.31*	3.50*
lateraldorsal thalamus (LD)	2.03	3.42*	3.49*
pretectal nucleus (APTD)	2.06	2.95*	3.39*
CA3	2.39	3.29*	3.00*
CA1	1.22	1.89	1.60
amygdalopiriform (Apir)	3.88	3.50	3.17
	5 minute SE		
Brain Region	**control n=8**	**150 mg/kg n=8**	**300 mg/kg n=8**
parietal cortex (Par1)	3.00	3.42	2.25
occipital cortex (Oc)	2.78	3.20	2.20
perirhinal cortex (PRh)	3.54	3.76	2.32*
piriform cortex (Pir)	3.97	4.00	3.43
cortical amygdala (Co)	4.00	4.00	3.57*
medial amygdala (BM)	3.41	3.88	3.50
lateral amygdala (La)	3.74	4.00	3.80
mediodorsal thalamus (MD)	3.05	3.93*	3.97*
lateraldorsal thalamus (LD)	3.15	3.99*	4.00*
pretectal nucleus (APTD)	2.71	3.41*	3.65*
CA3	2.80	3.64*	3.62*
CA1	1.52	2.47	2.59*
amygdalopiriform (Apir)	4.00	3.63	3.88

Nicotinamide induced minor neuroprotection in the cholinergic convulsions. 300 mg/kg nicotinamide exposure treatment induced significant reductions in necrosis lesions scores in the parietal, occipital and perirhinal cortices (Table 2). Administered 5 minutes following SE onset 300 mg/kg significantly reduced the neuropathology scores in the perirhinal cortex and cortical amygdala. The neuroprotection induced by the nicotinamide exposure treatment is illustrated in greater detail in Figure 11. All neuropathology data includes the necrosis scores of the one rat in each nicotinamide group that did not experience SE.

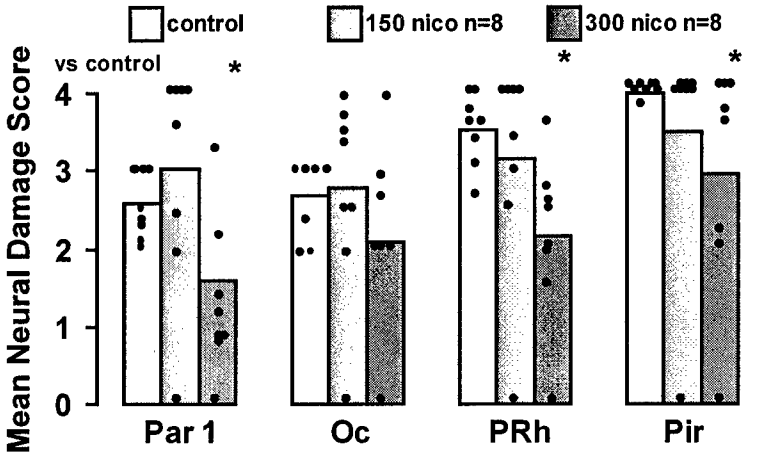
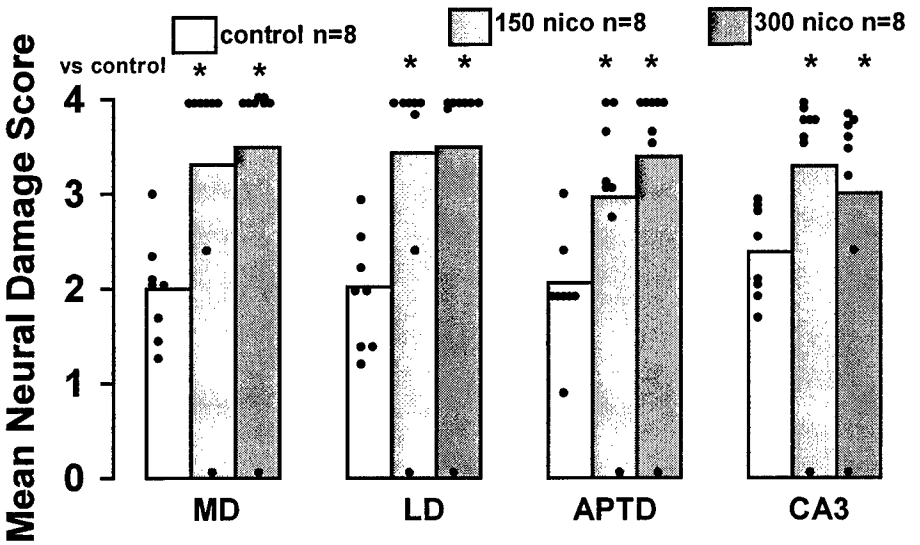


Figure 11. Graphic presentation of the neuroprotection induced by nicotinamide administered as exposure treatment. Both individual neuropathology scores and the group average are depicted. Asterisk (*) indicates significant difference from the corresponding Li-pilo control group as determined by Kruskal-Wallis H-test. Abbreviations are as indicated in Table 2.

Unfortunately, nicotinamide also exacerbated the cholinergic convulsion-induced neuropathology in thalamic and hippocampal regions. Regardless of time of administration, both doses of nicotinamide significantly increased the neuropathology scores in the mediodorsal thalamus, lateraldorsal thalamus, pretectal nucleus and CA3 region of the hippocampus (Table 2). The increased neuropathology induced by the nicotinamide exposure is illustrated in greater detail in Figure 12.

Figure 12. Graphic presentation of the exacerbated neuropathology induced by nicotinamide administered as exposure treatment. Both individual neuropathology scores and the group average are depicted. Asterisk (*) indicates significant difference from the corresponding Li-pilo control group as determined by Kruskal-Wallis H-test.



Nicotinamide produced neuroprotection in temporal regions of rats experiencing Li-pilo SE. The effect was dose related, as 300 mg/kg was neuroprotective while 150 mg/kg was not. In general, the neuroprotective effect of nicotinamide appears to be dose dependent as 500 mg/kg nicotinamide was neuroprotective in permanent ischemic stroke while either 250 or 750 mg/kg were ineffective (Sakakibara et al., 2000). Multiple administrations may also be of value as two repeated 500 mg/kg nicotinamide doses over a two week period significantly improved the neurological deficit induced by transient ischemic stroke (Maynard et al., 2001). Taken together these data would indicate that the neuroprotective effects of nicotinamide are dependent on dose and time of administration.

We suggest that the full neuroprotectant activity of nicotinamide has yet to demonstrated in cholinergic convulsions. This is because the neuroprotectant effects of nicotinamide are dose dependent and higher doses than tested here are effective in ischemic stroke (Ayoub et al., 1999;Sakakibara et al., 2000; Toshihiko et al., 2000). A multiple dose regimen should also be considered as repeated doses of nicotinamide are neuroprotective in ischemic stroke (Maynard et al., 2001). Finally, the PARP inhibitor benzamide is neuroprotective in rat temporal regions during soman SE (Meier et al., 1999). We feel that higher doses administered over a greater period of time with neuropathological and neurobehavioral outcomes evaluated over a longer survival period would be valuable.

Nicotinamide had no effect on neurological deficit as determined by spontaneous activity 24 hours after acute Li-pilo SE. However, repeated nicotinamide doses did improve neurological scores to the level of control animals in ischemic stroke (Maynard et al., 2001). As discussed in previous progress reports, the neurological deficit one day following 3 hours of Li-pilo SE may be too severe for any drug treatment to induce significant effects on neurological or cognitive deficits. We suggest employing survival periods of up to 6 weeks while assessing multiple parameters including neuropathology, behavior and epileptogenesis.

Nicotinamide has been reported to exert anticonvulsant activity (Kryzhanoskii et al., 1980; Braslavskii et al., 1982). In the present study, nicotinamide administered as exposure treatment prevented SE in 1 of 8 rats at both 150 and 300 mg/kg doses. In addition, the 150 mg/kg nicotinamide exposure dose induced a small but significant decrease in AD duration.

As previously reported for 1-aminocyclopropanecarboxylic acid (ACPC), mefenamate and PBN, nicotinamide enhanced neuropathology in thalamic regions (Peterson et al., 2004). These regions are critical to limbic SE (Bertram et al., 2000; Zhang and Bertram, 2002) and are the only regions consistently damaged by Li-pilo SE (Bertram and Scott, 2000). Interestingly, of all the drugs tested in this project, it is the compounds that produce neuroprotection in cortical areas that enhance thalamic neuropathology. D-cycloserine was the exception that did not protect the cortex and did not alter thalamic pathology (Peterson et al., 2004). We speculate that neuroprotection in cortical areas alters the balance of neural activity in the thalamo-cortical circuits such that thalamic tissue receives a greater deficit. It may be necessary to limit the search for nerve agents medical countermeasures to drugs that protect all brain regions.

Nicotinamide acts as a PARP inhibitor. It is also a precursor of NAD^+ which would reverse the energy depletion resulting from the ATP conversion to NAD^+ during SE. In ischemic stroke, nicotinamide neuroprotection was not associated with alterations in tissue NAD^+ (Sadanaga-Akiyoshi et al., 2003). Likewise, nicotinamide-induced vasodilation was not correlated with neuroprotection (Sadanaga-Akiyoshi et al., 2003). Those results suggest nicotinamide neuroprotection is due to PARP inhibition.

N-acetylcysteine

NMDA receptors induce NFκB activation as a mechanism of neurotoxicity. NFκB is a transcription factor that is maintained inactive in the cytosol by a specific inhibitor (IκB). Various signals trigger the dissociation of NFκB from IκB, which activates the translocation of NFκB to the nucleus where it induces the expression of numerous genes (Hensley et al., 1997; Michaelis, 1997; Grilli and Memo, 1999). Some of the signals that activate brain NFκB translocation include inflammatory cytokines (TNFα, IL-1), phorbol esters, oxidative stress and ROS (Grilli and Memo, 1999; Michaelis, 1997). Although some of the genes regulated by NFκB may be neuroprotective, most of the genes expressed are believed to be deleterious to neurons (Grilli and Memo, 1999). The deleterious genes regulated by NFκB include those responsible for the expression of

inflammatory cytokines, chemokines, iNOS (inducible NOS), cyclooxygenase-2 and manganese-superoxide dismutase (Grilli and Memo, 1999; Michaelis, 1997).

Glutamate also induces NFκB activation. The activation is mediated by NMDA receptors as NMDA antagonists block NFκB activation by glutamate in cerebellar granule cells (Guerrini et al., 1995). The NFκB activation in primary neurons is also inhibited by antioxidants, suggesting that the NMDA receptor activation of NFκB is mediated by ROS (Kaltschmidt et al., 1995). In intact rats, MK-801 blocks hypoxia-induced increases in NFκB activity, again supporting a NMDA receptor mechanism (Gozal et al., 1998). Kainate, pentylenetetrazol and most importantly, lithium-pilocarpine-induced seizures activate NFκB in rats supporting a connection between NFκB activation and seizure activity (Prasad et al., 1994; Rong and Baudry, 1996; Unlap and Jope, 1995).

N-acetylcysteine also acts as a ROS scavenger and as a precursor for glutathione (δ-glutamylcysteinylglycine) which in turn acts as a ROS scavenger (Staal et al., 1990). These antioxidant properties are expected to counteract the ROS component of NMDA receptor-mediated toxicity and contribute to the neuroprotective effect of N-acetylcysteine.

N-acetylcysteine was proposed for study because as a NFκB inhibitor it inhibits deleterious gene expression that contributes to the NMDA recepetor-mediated neurotoxicity. In addition, N-acetylcysteine has antioxidant properties that are expected to contribute to neuroprotection from NMDA receptor-mediated toxicity. It is readily available, easy to administer and has low toxicity. We hypothesize that while N-acetylcysteine may not have anticonvulsant activity it will reduce the seizure-induced neuropathology and neurological deficit.

As tested under our experimental conditions, N-acetylcysteine (NAC) exacerbated the deleterious effects of Li-pilo SE. NAC had no effect on the onset or duration of SE. Four of 8 rats administered 250 mg/kg NAC as exposure treatment did not survive the 24 hour period prior to the scheduled sacrifice. The neurological deficit as determined by spontaneous activity was significantly greater in the group administered 250 mg/kg NAC 5 minutes following SE onset. The neuropathology of Li-pilo SE was only enhanced by NAC.

NAC administered as exposure treatment had no effect on the onset of SE (Figure 13).

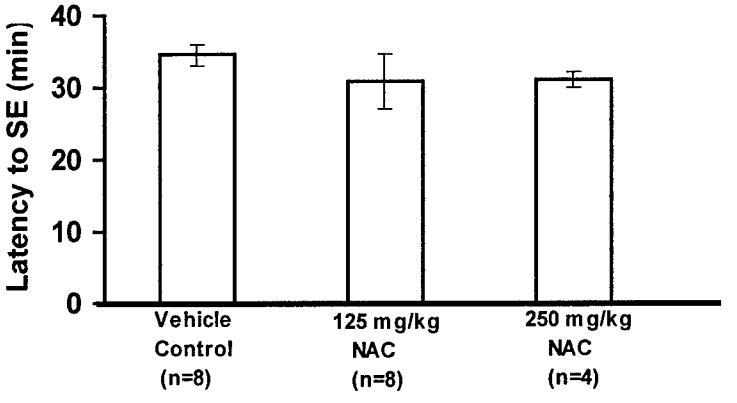


Figure 13. Latency to SE onset was the time from pilocarpine administration to the start of SE as defined by ECoG spiking. 4 of 8 rats treated with 250 mg/kg NAC did not survive overnight. Only data from the survivors is shown (one way ANOVA).

NAC had no significant effect on ECoG afterdischarge duration (Figure 14).

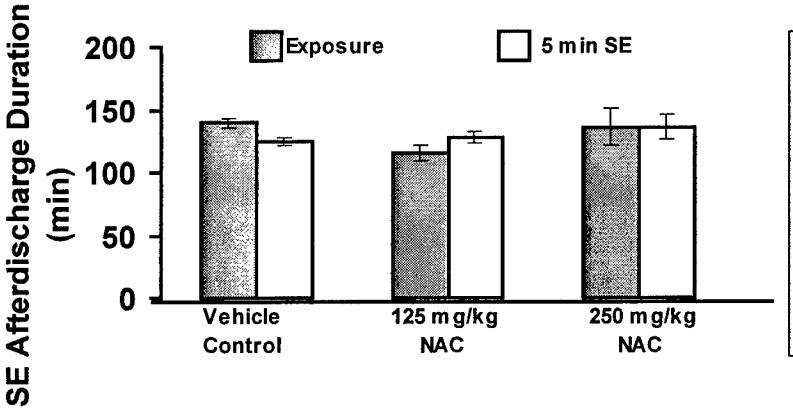


Figure 14. SE afterdischarge duration defined as the period of continuous high amplitude ECoG spiking. NAC induced no significant changes (one way ANOVA).

Administration of NAC as exposure treatment had no effect on distance traveled (DT) or resting time (RT) as determined in the spontaneous activity assessment (Figure 15).

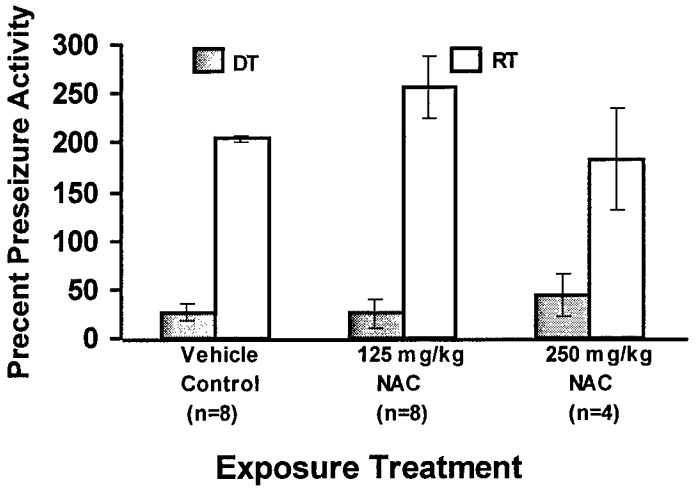


Figure 15. Effects of Li-pilo SE on spontaneous activity expressed as a percent of preseizure activity. Spontaneous activity was determined for 10 minutes in an activity monitor both 24 hours before and after pilocarpine administration. NAC exposure treatment had no effect on the neurological deficit (one way ANOVA).

Administration of NAC following 5 minutes of Li-pilo SE significantly affected spontaneous behavior (Figure 16). 125 mg/kg NAC increased the distance traveled as compared to the vehicle control and 250 mg/kg group which may indicate an improvement in neurological function. However, the 250 mg/kg NAC dose significantly increased the resting time as compared to the vehicle control and 125 mg/kg NAC groups. The significant increased in resting time and decrease in distance traveled as compared to the 125 mg/kg NAC group, suggests that the animals in the 250 mg/kg group were seriously injured and moved very little when tested. This indicates that 250 mg/kg NAC exacerbated the Li-pilo neurological deficit.

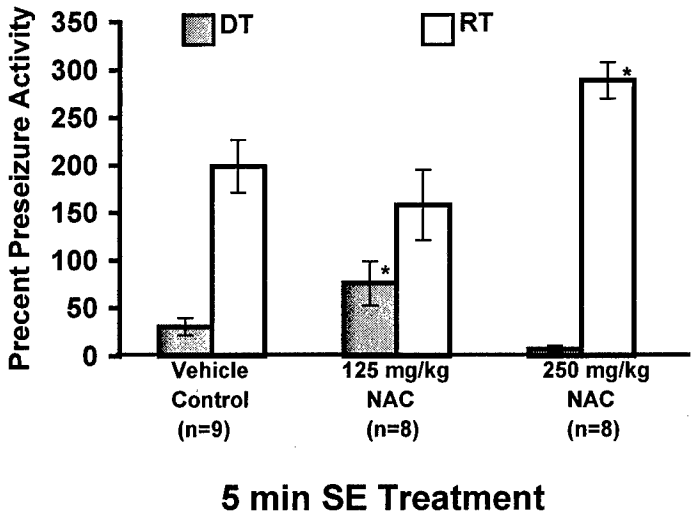


Figure 16. NAC administration following 5 minutes of Li-pilo-induced SE altered the neurological deficit as measured by distance traveled (DT) and resting time (RT). * indicates significant difference from both of the corresponding groups (one way ANOVA). Animals in the 250 mg/kg NAC group displayed very little movement 24 hours following Li-pilo SE.

Twenty-four hours following pilocarpine the animals were sacrificed and perfusion fixed for histological analysis of SE-induced neuronal damage. The results are presented in Table 3.

Table 3. Mean neural damage scores following N-acetylcysteine (NAC) treatment for brain structures in the region –0.8 to -4.8 mm from bregma. * indicates significant difference from vehicle control groups determined by Kruskal-Wallis H-test. In no case was the neuropathology reduced by NAC.

Effects of NAC on neural damage

	Exposure treatment		
Brain region	**control n=8**	**125 mg/kg n=8**	**250 mg/kg n=4**
parietal cortex (Par1)	2.70	3.46	3.30
occipital cortex (Oc)	2.69	3.56*	3.11
perirhinal cortex (PRh)	3.60	3.82	3.53
piriform cortex (Pir)	4.00	3.99	3.98
cortical amygdala (Co)	3.91	4.00	4.00
medial amygdala (BM)	3.25	3.99*	3.83
lateral amygdala (La)	3.72	3.96	3.83
mediodorsal thalamus (MD)	2.28	4.00*	4.00*
lateraldorsal thalamus (LD)	2.31	4.00*	4.00*
pretectal nucleus (APTD)	2.13	3.70*	3.85*
CA3	2.53	3.90*	3.62*
CA1	1.33	2.36*	2.85*
amygdalopiriform (Apir)	4.00	4.00	4.00
	5 minute SE		
Brain Region	**control n=9**	**125 mg/kg n=8**	**250 mg/kg n=8**
parietal cortex (Par1)	3.00	3.20	3.59
occipital cortex (Oc)	2.80	3.29	3.18
perirhinal cortex (PRh)	3.59	3.85	3.96
piriform cortex (Pir)	3.97	4.00	3.99
cortical amygdala (Co)	4.00	4.00	4.00
medial amygdala (BM)	3.45	3.92*	3.90*
lateral amygdala (La)	3.77	3.97	3.98
mediodorsal thalamus (MD)	3.09	4.00*	4.00*
lateraldorsal thalamus (LD)	3.20	4.00*	4.00*
pretectal nucleus (APTD)	2.74	3.88*	3.53*
CA3	2.81	3.66*	3.63*
CA1	1.46	2.34*	3.00*
amygdalopiriform (Apir)	4.00	4.00	4.00

NAC consistently enhanced the neuropathology observed in the medial amygdala, mediodorsal thalamus, lateral dorsal thalamus, pretectal nucleus, CA3 region of hippocampus and CA1 region of the hippocampus. In addition, 125 mg/kg NAC exposure treatment exacerbated neuropathology in the occipital cortex (Table 3).

In Li-pilo SE, NAC had no anticonvulsant activity, enhanced the neurological deficit and exacerbated the neuropathology without providing neuroprotection in any region. This is surprising as NAC reduces ischemic lesion volume and improves neurological scores in a variety of experimental models of stroke (Carrol et al., 1998; Sekhon et al., 2003; Khan et al., 2004). The 125 and 250 mg/kg i.p. NAC doses used in the Li-pilo SE by this study bracket the 150 mg/kg i.p. dose employed in the stroke studies (Carroll et al., 1998; Sekhon et al., 2003; Khan et al., 2004) which rules out the possibility of an ineffective dose. However, those ischemic stroke studies employed multiple dose regimens which may account for the apparent inactivity in the present study.

NAC is anticonvulsant in a congenital myoclonic epilepsy known as Unverricht-Lundborg disease (Edwards et al., 2002; Ben-Menachem et al., 2000). This condition is hypothesized to result from alterations in superoxide dismutase levels leading to a free radical mediated seizure mechanisms. NAC is proposed to act as an antioxidant to reduce the free radical load and inhibit the seizure activity in Unverricht-Lundborg. The NAC failure to inhibit seizure activity in our experiments suggests that free radicals themselves do not mediate the actual seizure activity in Li-pilo SE.

NAC acts as an antioxidant, a glutathione precursor and a NFκB inhibitor (Staal et al., 1990). Given the NAC failure to improve Li-pilo SE outcome we suggest that NFκB inhibition is not a likely mechanism for neuroprotection in cholinergic convulsions. In fact, the NFκB inhibition was associated with increased neuropathology and neurological deficit which may indicate a neuroprotective effect for NFκB activity. Another free radical scavenger/antioxidant tested in this project, PBN, has been shown to induce neuroprotection in Li-pilo SE. This suggests there are differences in the species of free radicals that are scavenged by PBN and NAC.

Given the widespread use in the human population and the reported neuroprotection in models of ischemic stroke, NAC seemed an ideal candidate to test as medical countermeasure to OP nerve agent exposure. However, based on the present negative results we do not recommend any further evaluation of NCA in the treatment of SE.

PBN

A manuscript reporting the results of this study has been submitted to NeuroToxicology and is currently in press. A PDF version of that manuscript is attached in the Appendix of this report (Appendix Item 1). A brief description of the results is provided here.

α-phenyl-N-*tert*-butylnitrone (PBN) is neuroprotectant in models of neuronal injury (Yue et al., 1992; Oliver et al., 1990; Cao and Phillis, 1994; Li et al., 2001) including

epilepsy (Milatovic et al., 2001; He et al., 1997; Rauca et al., 2004; Rejchrtova et al., 2004). N-*tert*-butyl-α-(2 sulfophenyl) nitrone (S-PBN) is a sulfonated derivative of PBN that is more water-soluble but is also neuroprotective (Schulz et al., 1995a; Schulz et al., 1995b; Yang et al., 2000). The major difference is pharmacokinetic in that PBN has a plasma half-life of 3 hours and readily penetrates the blood-brain barrier (BBB) (Chen et al., 1990) while S-PBN has a plasma half-life of 9 minutes and poor BBB penetration (Marklund et al., 2001a; Yang et al., 2000). Despite these differences PBN and S-PBN were equally effective neuroprotectants in experimental models of neuronal injury (Yang et al., 2000; Marklund et al., 2001a; 2001b; 2002).

In a direct comparison between PBN and S-PBN in Li-pilo SE, PBN induced significant neuroprotection in temporal lobe regions while S-PBN did not. We propose that PBN was able to pass through the BBB in the temporal cortex region while S-PBN did not. We further propose that the free radical scavenging activity by PBN (Sen et al., 1994; Marklund et al., 2001a; Rauca et al., 2004) induced the neuroprotection observed in Li-pilo SE (Bruce & Baudry, 1995; Rong et al., 1999; Peterson et al., 2002).

Both PBN and S-PBN exacerbated the Li-pilo SE-induced neuropathology in the thalamus. We propose that this is due to the extensive increase in BBB permeability in the thalamus during Li-pilo SE (Leroy et al., 2003; van Eijsdenet et al., 2004) which would explain why both drugs induced an effect in this region. The mechanism by which PBN and S-PBN exacerbated the thalamic neuropathology is unknown but it is interesting that the mediodorsal thalamus plays a critical role in the development of acute limbic SE (Zhay & Bertram, 2002) and in the region that most consistently shows neuronal damage in limbic SE (Bertram & Scott, 2000).

We propose that PBN has potential as a neuroprotectant in the treatment of OP nerve agent-induced convulsions. Significant neuroprotection was demonstrated in Li-pilo SE in the present study. Rejchrtova et al. (2005) have shown PBN neuroprotection in Li-pilo seizures induced in postnatal day 25 rats. This collaborates previous reports of PBN neuroprotection in experimental models of neuronal injury (Yue et al., 1992; Oliver et al., 1990; Cao & Phillis, 1994; Li et al., 2001) and experimental models of epilepsy (He et al., 1990; Milatovic et al., 2001; Rauca et al., 2004). The neuroprotection in Li-pilo SE resulted from a single administration of PBN. Given that repeated PBN administration paradigms have been successful in other models of brain injury (Marklund et al. 2001b; Li et al., 2001; Rejchrtova et al., 2005) we expect that a repeated PBN administration paradigm would result in even greater neuroprotection in SE.

ACPC and DCS

The results of the 1-aminocyclopropanecarboxylic acid (ACPC) and D-cycloserine (DCS) study have been reported previously. A report of the results as published in NeuroToxicology is attached in the Appendix (Appendix Item 2). A concluding statement is provided here.

ACPC and DCS were tested as partial agonists of the strychnine-insensitive site of the NMDA receptor (Henderson et al., 1990; Watson and Lanthorn, 1990) that are known to

induce anticonvulsant activity. DCS is anticonvulsant in maximal electroshock (Peterson, 1992), kindled amygdaloid seizures (Loscher et al., 1994) and kainate SE (Baran et al., 1994). ACPC is anticonvulsant in NMDA-induced seizures (Skolnick et al., 1989) and audiogenic seizures (Smith et al., 1993). ACPC is also neuroprotective in experimental models of ischemic stroke (Fossom et al., 1995) and spinal cord injury (Long and Skolnick, 1994).

ACPC induced significant neuroprotection in Li-pilo SE. Administered 5 minutes following SE onset, ACPC reduced by half the neural damage scores induced by 3 hours SE in the parietal, occipital and CA1 region of the hippocampus. The neuroprotection occurred in spite of continuing SE that was unaffected by ACPC. Interestingly ACPC exacerbated the SE-induced neuropathology found in the thalamus. The difference in the ACPC effects between the thalamus and cortical regions may be the predominance of NMDAR2A receptors in the cortex (Ishii et al., 1993) where ACPC has neuroprotective NMDA antagonist activity (Sheinin et al., 2002). NMDAR2B and 2C receptors predominate in the thalamus (Ishii et al., 1993) where ACPC exerts more glycine agonist activity (Ishii et al., 1993) which would enhance NMDA receptor toxicity (Nahum-Levy et al., 1999; Sheinin et al., 2002). Thus it appears that regional NMDA receptor subtype distribution affects the ACPC induced effect on SE-induced neuropathology.

Interestingly, DCS had neither anticonvulsant nor neuroprotectant activity in Li-pilo SE whereas it is reported to have significant anticonvulsant activity in kainate SE (Baran et al., 1994). This underscores the differences in mechanism of action between the various models of limbic SE (Ferkany et al., 1982; McDonough and Shih, 1997) and indicates kainate is not an adequate model of OP nerve agents. Because glutamate antagonists inhibit both kainate (Clifford et al., 1998) and Li-pilo convulsions (Ormandy et al., 1989) we propose that different subsets of NMDA receptors are involved in the two seizure types and that DCS is active only with NMDA receptors involved in kainate SE (Ishii et al., 1993; Sheinin et al., 2001).

ACPC induced significant neuroprotection when administered at the onset of Li-pilo-induced SE. Due to the short duration of action of ACPC in rats (Smith et al., 1993), we propose that repeated ACPC doses would enhance neuroprotection. The heterogeneity of NMDA receptor expression may explain regional differences in ACPC neuroprotection as well as the failure of DCS to affect Li-pilo SE.

Completed Tasks Outside of the Approved Statement of Work

Propofol

Propofol was not included in the original proposal as a possible countermeasure to OP nerve agent exposure. During initial trials with control animals we found that rats did not survive the required 24 hours following Li-pilo SE induction. Propofol was tested as a means to improve 24-hour survival. When propofol was administered after 3 hours of Li-pilo SE, we found that the animals still experienced significant neuropathology against which the candidate neuroprotectants and anticonvulsants could be tested. In addition, the animals also survived 24 hours. Upon further experimentation we found that propofol itself induce significant anticonvulsant and neuroprotectant effects. Because of limited

resources only a small pilot study was conducted. The results of that study were presented at the Bioscience 2004 meeting and a copy of the abstract and manuscript is provided in the Appendix (Appendix Item 3). A brief description of the study outcome is provided here.

Propofol, 55 mg/kg i.p., administered either 5 minutes or 3 hours following the onset of SE inhibited all seizure activity. Following propofol administration the animals appeared anesthetized and the ECoG was nearly isoelectric. Upon recovery from anesthesia the animals that had been administered propofol 5 minutes following SE onset did not experience any further seizure activity and demonstrated no neuropathology when sacrificed 24 hours later. Seizure activity did return in animals administered propofol following 3 hours SE and the animals exhibited significant neuropathology and macroscopic lesion in the piriform cortex. However, when an additional 20 mg/kg propofol was administered 2 hours following the initial 3 hour post SE 55 mg/kg propofol dose, the macroscopic piriform lesions were significantly reduced or absent. Based on these finding we concluded that propofol induce significant anticonvulsant and neuroprotectant activity.

Following the presentation of this data to the Bioscience 2004 meeting, Dr. McDonough's laboratory at USAMRICD tested propofol in the guinea pig model of soman-induced SE. It appears that propofol is not anticonvulsant when tested in that model (McDonough, personal communication). It is also known that the guinea pig does not demonstrate macroscopic piriform lesions following limbic SE as is seen in rats. This underscores potential critical differences in the underlying mechanisms in guinea pig and rat SE. The guinea pig is generally considered the most relevant preclinical model when testing for nerve agent treatment. Thus there is some doubt as to the usefulness of propofol in treatment of nerve agent exposure.

KEY RESEARCH ACCOMPLISHMENTS

- Completed planned study of vehicle control.
- Completed planned study of mefenamate.
- Completed planned study of nicotinamide.
- Completed planned study of N-acetylcysteine.
- Completed planned study of a-phenyl-N-*tert*-butylnitrone (PBN)
- Completed planned study of 1-aminocyclopropanecarboxylic acid (ACPC).
- Completed planned study of D-cycloserine (DCS).
- Completed the entire Statement of Work.

REPORTABLE OUTCOMES

- Peterson, S.L., Purvis, R.S. and Griffith, J.W. Propofol Neuroprotection in Cholinergic Status Epilepticus. Bioscience 2004 Abstracts, Page 163.
- Peterson, S.L., Purvis, R.S. and Griffith, J.W. Differential Neuroprotective Effects of the NMDA Receptor-Associated Glycine Site Partial Agonists 1-Aminocyclopropanecarboxylic Acid (ACPC) and D-Cycloserine in Lithium-Pilocarpine Status Epilepticus. NeuroToxicology 25:835-847, 2004.

- Peterson, S.L., Purvis, R.S. and Griffith, J.W. Comparison of Neuroprotective Effects Induced by α-phenyl-N-*tert*-butyl nitrone (PBN) and N-*tert*-butyl-α-(2 sulfophenyl) nitrone (S-PBN) in Lithium-Pilocarpine Status Epilepticus NeuroToxicology, In Press.
- Submitted proposal entitled "Propofol Neuroprotection in Cholinergic Status Epilepticus" to Department of Defense Congressionally Directed Medical Research Programs (CDMRP). Log number PR043077. Steven Peterson Principal Investigator. Requesting $1,923,587 total costs for 4 years. Project was not funded.
- Submitted proposal entitled "Cholinergic Model of Acute Antiepileptogenic Therapy" to NIH in response to RFA-NS-04-002. Assignment number R21 NS049619-01. Steven Peterson Principal Investigator. Requesting $250,000 direct costs for 2 years. Project was not funded.

Bibliography of publications

1. Peterson, S.L., Purvis, R.S. and Griffith, J.W. Propofol Neuroprotection in Cholinergic Status Epilepticus. Bioscience 2004 Abstracts, Page 163.
2. Peterson, S.L., Purvis, R.S. and Griffith, J.W. Differential Neuroprotective Effects of the NMDA Receptor-Associated Glycine Site Partial Agonists 1-Aminocyclopropanecarboxylic Acid (ACPC) and D-Cycloserine in Lithium-Pilocarpine Status Epilepticus. NeuroToxicology 25:835-847, 2004.
3. Peterson, S.L., Purvis, R.S. and Griffith, J.W. Comparison of Neuroprotective Effects Induced by α-phenyl-N-*tert*-butyl nitrone (PBN) and N-*tert*-butyl-α-(2 sulfophenyl) nitrone (S-PBN) in Lithium-Pilocarpine Status Epilepticus NeuroToxicology, In Press.

List of Personnel Receiving Pay From Research Effort

1. Steven Peterson, Professor, UNM College of Pharmacy
2. Rebecca Purvis, Research Technician, UNM College of Pharmacy
3. James Griffith, Professor, Penn St. Milton S. Hershey Medical Center

CONCLUSIONS

As weapons of mass destruction, organophosporus (OP) nerve agents pose a continuing threat to American military and civilian populations. Nerve agents produce a generalized convulsive seizure state that is characterized by the same clinical features as status epilepticus (SE). Survivors of SE are subject to chronic neuropathology including epileptic brain damage, neurological and cognitive deficits as well as the development of epilepsy (Lothman & Bertram, 1993; Treiman, 1993). Both long-term behavioral and neurological deficits induced by nerve agents have been extensively documented in experimental animals (Geller et al., 1985; Raffaele et al., 1987; McDonough et al., 1986; Ingrid et al., 1992; McDonough & Shih, 1997; McDonough et al., 1998; Filliat et al., 1999). The SE induced by cholinergic convulsions in experimental animals results in spontaneous recurrent seizures that require a latent period of several weeks to fully develop. This period of epileptogenesis models human epilepsy, as the seizures are both paroxysmal and recurrent. In addition, chronic cognitive deficits result from cholinergic convulsions in experimental animals (Hort et al., 1999; Harrigan et al, 1991; Balakrisnan

et al., 2001; Raffaele et al., 1987; McDonough et al., 1986; Modrow et al., 1989; Ingrid et al., 1992).

Li-pilo (Jope et al., 1986; Morrisett et al., 1998) and soman (Shih et al., 1997; McDonough & Shih, 1997) produce convulsions that are relatively refractory to drug therapy with the additional confound that the longer SE continues the more difficult pharmacological treatment becomes (Walton & Treiman, 1991; Jones et al., 2002). Benzodiazepines are the current treatment used specifically for the seizures induced by OP nerve agents. Diazepam and midazolam are most effective when administered at seizure onset but this may not be practical in the field exposure setting. Presently there is little medical recourse should midazolam fail to prevent the OP nerve-agent SE. A medical countermeasure is needed that will be effective in the late stages of medical emergency SE, commonly after 2-3 hours of seizure activity. The results of project DAMD17-01-1-0794 indicate that 1-aminocyclopropanecarboxylic acid (ACPC) and α-phenyl-N-*tert*-butyl nitrone (PBN) produce neuroprotection even during unabated SE. We would suggest that these neuroprotectants be investigated further as medical countermeasures to OP nerve agent toxicity.

We recommend that both ACPC and PBN be tested further using multiple dose treatment regimens. Both drugs produced neuroprotectant effects following a single acute administration in Li-pilo SE. ACPC has a relatively short duration of action (Smith et al., 1993) and would be expected to produce greater neuroprotection with multiple administrations. Repeated PBN administration paradigms have been successful in other models of brain injury (Marklund et al., 2001b; Li et al., 2001; Rejchrtova et al., 2004) and we propose that repeated PBN administration would result in greater neuroprotection in SE.

We further recommend that ACPC and PBN be tested for activity against the long-term cognitive and epileptogenic effects of cholinergic SE. Neuroprotection implies that the brain has been protected from seizure-induced neuronal damage and pharmacological treatment protects the neuronal integrity and overall brain morphology following cholinergic convulsions (Fujikawa et al., 1994; Fujikawa, 1995; Andre et al., 2001; Rigoulot et al., 2003). However, the ultimate concern is whether brain function has been preserved and it has recently been shown that preservation of neuronal integrity and morphology following SE does not prevent spontaneous recurrent seizure development (Ebert et al., 2002; Brandt et al., 2003). It is probably true that "morphological neuroprotection" does not guarantee "functional neuroprotection" and that both must be assessed when evaluating pharmacological treatment of SE. We suggest that future studies of neuroprotectant treatment evaluate both spontaneous recurrent seizure development (epileptogenesis) and cognitive function as much as 6 weeks following the OP-induced SE.

As described in previous progress reports and presented at the Bioscience 2004 meeting in May 2003, the general anesthetic propofol produced significant anticonvulsant and neuroprotectant activity in the Li-pilo SE model in rats. When tested in the guinea pig soman model, propofol was found to have no anticonvulsant activity (McDonough,

personal communication). Further, the macroscopic lesions induced in the rat temporal lobe by cholinergic SE are not found in guinea pigs so the neuroprotectant activity of propofol in guinea pigs is not known at this time. Given that soman-induced SE in the guinea pig is the premier pre-clinical model for primate OP nerve agent response, the results suggest propofol is not as effective as the rat Li-pilo model suggested. Nevertheless, during the course of the propofol experiments it became apparent that anesthesia and hypothermia are critical factors in neuroprotection during SE. We suggest that anesthesia and hypothermia be investigated as potential medical countermeasures to OP nerve agents. The investigators are interested in pursuing anesthesia and hypothermia studies in a rat model of soman-induced SE.

REFERENCES

Andre, V., Rogoulot, M-A., Koning, D., et al., 2001. Alterations of hippocampal GABAergic system contribute to development of spontaneous recurrent seizures in the rat lithium-pilocarpine model of temporal lobe epilepsy. Hippocampus 11:452-468.

Asanuma, M., Nishibayashi-Asanuma, S., Miyazaki, I., et al., 2001. Neuroprotective effects of non-steroidal anti-inflammatory drugs by direct scavenging of nitric oxide radicals. J. Neurochem. 76:1895-1904.

Ayoub, I.A., Lee, E.J., Ogilvy, C.S., et al., 1999. Nicotinamide reduces infarction up to two hours after the onset of permanent focal cerebral ischemia in Wistar rats. Neurosci Lett. 259: 21-24.

Balakrishnan, S., Nidhi, G., Pandhi, P., 2001. Effect of duration of pilocarpine-induced status epileptiucs on subsequent cognitive function in rats. Methods and Findings in Experimental and Clinical Pharmacology 23(2):85-87.

Baran, H., Vass, K., Lassmann, H., Hornykiewica, O., 1994. The cyclooxygenase and lipowygenase inhibitor BW755C protects rats against kainic acid-induced seizures and neurotoxicity. Brain Res 646:201-206.

Bertram, E.H., Scott, C., 2000. The pathological substrate of limbic epilepsy: neuronal loss in the medial dorsal thalamic nucleus as the consistent change. Epilepsia 41(S6):S3-S8.

Bertram, E.H., Mangan, P.S., Zhang, D., et al., 2001. The midline thalamus: alterations and a potential role in limbic epilepsy. Epilepsia 42(8):967-978.

Brandt, C., Potschka, H., Loscher, W., et al., 2003. N-Methyl-D-aspartate receptor blockade after status epilepticus protects against limbic brain damage but not against epilepsy in the kainate model of temporal lobe epilepsy. Neuroscience 118:727-740.

Braslavskii, V.E., Shchavelev, V.A., Kryzhanoskii, G.N, et al., 1982. Effect of nicotinamide on focal and generalized epileptic activity in the cerebral cortex. Biull Eksp Biol Med 94:39-42.

Bruce, A.J., Baudry, M., 1995. Oxygen free radicals in rat limbic structures after kainite-induced seizures. Free Radic Biol Med. 18(6): 993-1002.

Cao, X. and Phillis, W., 1994. alpha-phenyl-tert-butyl-nitrone reduces cortical infarct and edema in rats subjected to focal ischemia. Brain Res. 644: 267-272.

Chen, Q., Olney, J.W., Lukasiewicz, P.D., et al., 1998. Fenamates protect neurons against ischemic and excitotoxic injury in chick embryo retina. Neurosci Lett 242:163-166.

Chen, G.M., Bray, T.M., Janzen, E.G., et al., 1990. Excretion, metabolism and tissue distribution of a spin trapping agent, alphs-phenyl-N-tert-butyl-nitrone (PBN) in rats. Free Radic Res Commun 9(3-6): 317-323.

Clifford, D.B., Olney, W., Maniotis, A., et al., 1987. The functional anatomy and pathology of lithium-pilocarpine and high-dose pilocarpine seizures. Neuroscience 23(3): 953-968.

Cosi, C., Suzuki, H., Milani, D., et al., 1994. Poly(ADP-ribose) polymerase: early involvement in glutamate-induced neurotoxicity in cultured cerebellar granule cells. J Neurosci Res 39:38-46.

Cosi, C., Chopin, P., Marien, M., 1996. Benzamide, an inhibitor of poly(ADP-ribose) polymerase, attenuates methamphetamine-induced dopamine neurotoxicity in the C57B1/6N mouse. Brain Res. 735:343-348.

Ebert, U., Brandt, C., Loscher, W., 2002. Delayed sclerosis, neuroprotection and limbic epileptogenesis after status epilepticus in the rat. Epilepsia 43(S5):86-95.

Eliasson, M.J., Sampei, K., Mandir, A.S., et al., 1997. Poly(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia. Nature Medicine 3: 1089-1095.

Filliat, P., Baubichon, D., Burckhart, M-F., et al., 1999. Memory impairment after soman intoxication in rat: correlation with central neuropathology. Improvement with anticholinergic and antiglutamatergic therapeutics. Neurotox. 20(4):535-550.

Fossom, L.H., Basile, A.S., Skolnick, P., 1995a. Sustained exposure to 1-aminocyclopropanecarboxylic acid, a glycine partial agonist, alters N-methyl-D-aspartate receptor function and subunit composition. Mol Pharmacol. 48(6): 981-987.

Fujikawa, E.G., Daniels, A.H., Kim, J.S., 1994. The competitive NMDA receptor antagonist CGP 40116 protects against status epilepticus-induced neuronal damage. Epilepsy Res 17:207-219.

Fujikawa, D.G., 1995. Neuroprotective effect of ketamine administered after status epilepticus onset. Epilepsia 36(2):186-195.

Fujikawa, D.G., 1996. The temporal evolution of neuronal damage from pilocarpine-induced status epilepticus. Brain Res. 725:11-22.

Fujikawa, D.G., Sehinmei, S.S., Cai, B., 1999. Lithium-pilocarpine-induced status epilepticus produces necrotic neurons with internucleosomal DNA fragmentation in adult rats. Eur J Neurosci. 11:1605-1614.

Gale, E.A., 1996a. Molecular mechanisms of beta-cell destruction in IDDM: the role of micotinamide. Norm Res. 45(S1): 40-43.

Gale, E.A., 1996b. Theory and practice of nicotinamide trials in pre-type 1 diabetes. J Ped Endocrinol Metabolism 9: 375-379.

Geller, I., Harmann, R.J., Gause, E.M., 1985. Effects of subchronic administration of soman on acquisition of avoidance-escape behavior by laboratory rats. Pharmacol biochem Behav 23:225-230.

Gozal, E., Simakajornboon, N., Gozal, D., 1998. NF-κB induction during in vivo hypoxia in dorsocaudal brain stem of rat: effect of MK-801 and L-NAME. Amer Physiol Soc 85: 372-376.

Grilli, M, and Memo, M., 1999. Nuclear factor-κ-B/Rel proteins. Biochem Pharmacol 57:1-7.

Guerrini, L., Blasi, F., Denis-Donini, S., 1995. Synaptic activation of NF-κB by glutamate in cerebellar granule neurons in vitro. Proc Natl Acad Sci 92:9077-9081.

He, Q.P., Smith, M.L., Siejso, B.K., 1997. Necrosis of the substantia nigra, pars reticulate, in flurothyl-induced status epilepticus is ameliorated by the spin trap alpha phenyl-N-tert-butyl nitrone. Free Radic Biol Med 22(5): 917-922.

Henderson, G., Johnson, J.W., Ascher, P., 1990. Competitive antagonists and partial agonists at the glycine modulatory site of the mouse N-methyl-D-aspartate receptor. J Physiology 430:189-212.

Hensley, Tabatabaie, Stewart, Pye, et al., 1997. Nitric oxide and derived species as toxic agents in stroke, AIDS dementia and chronic neurodegenerative disorders. Chem Res Toxicol 10:527-532.

Harrigan, T., Peredery, O., Persinger, M., 1991. Radial maze learing deficits and mediodorsal thalamic damage in context of multifocal seizure-induced brain lesions. Behavioral Neuroscience 105(3):482-486.

Hort, J., Brozek, G., Mares, P., et al., 1999. Cognitive functions after pilocarpine-induced status epilepticus: changes during silent period precede appearance of spontaneous recurrent seizures. Epilepsia 40(9):1177-1183.

Ikonomidou-Turski, C., Cavalheiro, E.A., Turski, L., et al., 1988. Differential effects of non-steroidal anti-inflammatory drugs on seizures produced by pilocarpine in rats. Brain Res 462:275-285.

Ingrid, H.C., Philippens, H.M., Bert, P.C., 1992. Behavioral performance, brain histology, and EEG sequela after immediate combined atropine/diazepam treatment of soman-intoxicated rats. 42:711-719.

Ishii, T., Moriyoshi, K., Sugihara, H., Sakurada, K., et al., 1993. Molecular characterizatin of the family of the NMDA receptor subunits. J Biol Chem 268(4):2836-2843.

Jones, D.M., Esmaeil, N., Maren, S., et al., 2002. Characterization of pharmacoresistance to benzodiazepines in the rat Li-pilocarpine model of status epilepticus. Epilepsy Res 50:301-312.

Jope, R., Morrisett, R.A., Snead, O.C., 1986. Characterization of lithium potentiation of pilocarpine-induced status epilepticus in rats. Exp Neurol 91:471-480.

Kaltschmidt, C., Kaltschmidt, B., Baeuerle, P.A., 1995. Stimulation of ionotropic glutamate receptors activates transcription factor NF-κB in primary neurons. Proc Natl Acad Sci 92:9618-9622.

Kelly, J.J. and Auer, R.N., 2003. Mefenamate, an agent that fails to attenuate experimental cerebral infarction. Can. J. Neurol. Sci. 30:259-262.

Kim, H-C., Jhoo, W-K., Bing, G., et al., 2000. Phenidone prevents kainite-induced neurotoxicity via antioxidant mechanisms. Brain Res. 874:15-23.

Kryzhanovskii, G.N., Shandra, A.A., Makul'kin, R.F., et al., 1980. Effect of nicotinamide on epileptic activity in the cerebral cortex. Biull Eksp Biol Med 89:37-41.

Leroy, C., Roch, C., Koning, E., Namar, I.J., Nehlig, A., 2003. In the lithium-pilocarpine model of epilepsy, brain lesions are not linked to changes in blood-brain barrier permeability: an autoradiographic study in adult and developing rats. Exp Neurol 182:361-372.

Li, P.A., He, Q.P., Nakamura, L. and Csiszar, K., 2001. Free radical spin trap alpha-N-tert-butyl-nitron inhibits caspase-3 activation and reduces brain damage following a severe forebrain ischemic injury. Free Rad Biol Med. 31(10): 1191-1197.

Lo, E.H., Bosque-Hamilton, B.A., Meng, W., 1998. Inhibition of poly(ADP-ribose) polymerase reduction of ischemic injury and attenuation of N-methyl-D-aspartate-induced neurotransmitter dysregulation. Stroke 29:830-836.

Long, J.B. and Skolnick, P., 1994. 1-Aminocyclopropanecarboxylic acid protects against dynorphin A-induced spinal injury. Eur J Pharmacology 261: 295-301.

Loscher, W., Wlaz, P., Rundfeldt, C., et al., 1994. Anticonvulsant effects of the glycine/NMDA receptor ligands D-cycloserine and D-serine but not R-(+)-HA-966 in amygdala-kindled rats. Br J Pharmacol. 112:97-106.

Lothman, E.W., Bertram, E.H., 1993. Epileptogenic effects of status epilepticus. Epilepsia 34(S):59.

Marklund, N., Lewander, T., Clausen, F., 2001a. Effects of the nitrone radical scavengers PBN and S-PBN on *In Vivo* trapping of reactive oxygen species after traumatic brain injury in rats. J Cereb Blood Flow Metab 21: 1259-1267.

Marklund, N., Clausen, F., McIntose, T.K., et al., 2001b. Free radical scavenger posttreatment improves functional and morphological outcome after fluid percussion injury in the rat. J Neurotrauma 18(8): 821-832.

Marklund, N., Sihver, S., Langstrom, B., 2002. Effect of traumatic brain injury and nitrone radical scavengers on relative changes in regional cerebral blood flow and glucose uptake in rats. J Neurotrauma 19(10): 1139-1153.

Maynard, K.I., Ayoub, I.A., Shen, C, 2001. Delayed multidose treatment with nicotinamide extends the degree and duration of neuroprotection by reducing infarction and improving behavioral scores up to two weeks following transient focal cerebral ischemia in wistar rats. Ann. N Y Acad Sci 939:416-424.

McDonough, J.H., Smith, R.F., Smith, C.D., 1986. Behavioral correlates of soman-induced neuropathology: deficits in DRL acquisition. Neurobehav Toxicol Teratol 8(2):179-187.

McDonough, J.H., Shih, T-M., 1997. Neuropharmacological mechanisms of nerve agent-induced seizure and neuropathology. Neuroscience and Biobehavioral Reviews 21(5):559-579.

McDonough, J.H., Clark, T.R., Stone, T.W., et al., 1998. Neural lesions in the rat and their relationship to EEG delta activity following seizures induced by the nerve agent soman. NeuroToxicology 19(3):381-392.

Meier, H.L., Ballough, G.P., Forster, J.S., Filbert, M.G., 1999. Benzamide, a poly(ADP-ribose) polymerase inhibitor, is neuroprotective against soman-induced seizure-related brain damage. Annuals New York Academy of Sciences 890:330-335.

Michaelis, 1988. Molecular biology of glutamate receptors in the central nervous system and their role in excitotoxicity, oxidative stress and aging. Progress in Neurobiology 54:369-416.

Milatovic, D., Zivin, M., Gupta, R.C. and Dettbarn, W.D., 2001. Alterations in cytochrome c oxidase activity and energy metabolites in response to kainic-induced status epilepticus. Brain Res. 912:67-78.

Modrow, H.E., Jaax, N.K., 1989. Effect of soman exposure on the acquisitions of an operant alternation task. Pharmacol Biochem Behav 32:49-53.

Morriset, R.A., Jope, R.S., Snead, O.C., 1987. Effects of drugs on the initiation and maintenance of status epilepticus induced by administration of pilocarpine to lithium-pretreated rats. Exp Neurol 97:193-200.

Motte, J., da Silva Fernandes, M.J., Baram, T.Z., Nehlig, A., 1998. Spatial and temporal evolution of neuronal activation, stress and injury in lithium-pilocarpine seizures in adult rats. Brain Res. 798, 61-72.

Nahum-Levy, R., Fossom, L.H., Skolnick, P. and Benveniste, M., 1999. Putative partial agonist 1-aminocyclopropanecarboxylic acid acts concurrently as a glycine-site agonist and a glutamate-site antagonists as NMDA receptors. Mol Pharmacol 56:1207-1218.

Oliver, C.N., Starke-Reed, P.E., Stadtman, E.R., et al., 1990. Oxidative damage to brain proteins, loss of glutamine synthetase activity, and production of free radicals during ischemia/reperfusion-induced injury to gerbil brain. Proc Natl Acad Sci 87:5144-5147.

Ormandy, G.C., Jope, R.S., Snead, O.C., 1989. Anticonvulsant actions of MK-801 on the lithium-pilocarpine model of status epilepticus in rats. Exp. Neurol. 106, 172-180.

Partridge, L.D. and Valenzuela, C.F., 2000. Bock of hippocampal CAN channels by flufenamate. Brain Res. 867:143-148.

Peredery, O., Persinger, M.A., Parker, G., Mastrosov, L., 2000. Temporal changes in neuronal dropout following inductions of lithium/pilocarpine seizures in the rat. Brain Res. 881, 9-17.

Peterson, S.L., Schwade, N.D., 1993. The anticonvulsant activity of D-cycloserine is specific for tonic convulsions. Epilepsy Res. 15: 141-148.

Peterson, S.L., Morrow, D., Liu, S., Liu, K.J., 2002. Hydroethidine detection of superoxide production during the lithium-pilocarpine model of status epilepticus. Epilepsy Res 49:226-238.

Peterson, S.L., Purvis, R.S., Griffith, J.W., 2004. Differential neuroprotective effects of the NMDA receptor-associated glycine site partial agonists 1-aminocyclopropanecarboxylic acid (ACPC) and D-cycloserine in lithium-pilocarpine status epilepticus. NeuroToxicology 25:835-847.

Pieper, A.A., Verma, A., Zhang, J., et al., 1999. Poly(ADP-ribose) polymerase, nitric oxide and cell death. TiPS 20: 171-181.

Prasad, A.V., Pilcher, W.H., Joseph, S.A., 1994. Nuclear factor-κB in rat brain: enhanced DNA-binding activity following convulsant-induced seizures. Neurosci Lett 170: 145-148.

Racine, R., 1972. Modification of seizure activity by electrical stimulation: II. Motor seizure. Electroenceph Clin Neurophysiol 32:281-294.

Raffaele, K., Hughley, D., Wenk, F., et al., 1987. Long-term behavioral changes in rats following organophosphonate exposure. Pharmacol Biochem Behav 27:407-412.

Rauca, C., Wiswedel, I., Zerbe, R., Keilhoff, G., Krug. M., 2004. The role of superoxide dismutase and α-tocopherol in the development of seizures and kindling induced by pentylenetetrazol-influence of the radical scavenger α-phenyl-N-tert-butyl nitrone. Brain Res 1009:203-212.

Rejchrtova, J., Kubova, H., Druga, R., Mares, P., Folbergrova, J., 2005. Effects of a free radical scavenger N-tert-butyl-alpha-phenylnitrone (PBN) on short-term recovery of immature rats after status epilepticus. Physiol Res 54(2):215-227.

Rigoulot, M-A., et al., 2003. Prolonged low-dose caffeine exposure protects against hippocampal damage but not against the occurrence of epilepsy in the lithium-pilocarpine model in the rat. Epilepsia 44(4):529-535.

Rong, Y., Baudry, M., 1996. Seizure activity results in a rapid induction of nuclear factor-κB in adult but not juvenile rat limbic structures. J Neurochem. 67(2): 662-668.

Rong, Y., Doctrow, S.R., Tocco, G., et al., 1999. EUK-134, a synthetic superoxide dismutase and catalses mimetic, prevents oxidative stress and attenuates kainite-induced neuropathology. Proc Natl Acad Sci. 96, 9897-9902.

Sadanaga-Akiyoshi, F., Yao, H., Tanuma, S., et al., 2003. Nicotinamide attenuates focal ischemic brain injury in rats: with special reference to changes in nicotinamide and NAD^+ levels in ischemic core and penumbra. Neurochem Res 28(8):1227-1234.

Sakakibara, Y., Mitha, A.P., Ogilby, S., et al., 2000. Post-treatment with nicotinamide (vitamin B_3) reduces the infarct volume following permanent focal cerebral ischemia in female Sprague-Dawley and Wistar rats. Neurosci Lett 281:111-114.

Sen, S., Goldman, H., Morehead, M., Murphy, S., Phillis, J.W., 1994. α-phenyl-tert-butyl-nitrone inhibits free radical release in brain concussion. Free Radic Biol Med 16:685-691.

Schulz, J.B., Henshaw, D.R., Siwek, D., et al., 1995a. Involvement of free radicals in excitotoxicity in vivo. J Neurochem. 64: 2239-2247.

Schulz, J.B., Matthews, R.T., Jenkins, B.G., 1995b. Improved therapeutic window for treatment of histotoxic hypoxia with a free radical spin trap. J Cereb Blood Flow Metab 15: 948-952.

Sheinin, A., Shavit, S. and Benveniste, M., 2001. Subunit specificity and mechanism of action of NMDA partial agonist D-cycloserine. Neuropharm 41:151-158.

Sheinin, A., Nahum-Levy, R., Shavit, S. and Benveniste, M., 2002. Specificity of putative partial agonsit, ACPC for the rat NMDA receptor subunits. Neurosci Lett 317:77-80.

Shih, T-M., McDonough, J.H., Koplovitz, I., 1997. Evaluation of anticonvulsant drugs for soman-induced seizure activity. J Amer Coll Toxicol 15(S2):S43-S60.

Skolnick, P., Marvizon, J., Jackson, B., Monn, J., et al., 1989. Blockade of N-methyl-D-aspartate induced convulsions by 1-aminocyclopopanecarboxylates. Life Sci. 45: 1647-1655.

Smith, S.C., Al-Zubaidy, Z.A., Chapman, A.G., Meldrum, B.S., 1993. Excitatory amino acid antagonists, lamotrigine and BW 1003C87 as anticonvulsants in the genetically epilepsy-prone rat. Epilepsy Res 15:101-111.

Staal, F., Roederer, M., Herzenberg, L., Herzenberg L., 1990. Intracellular thiols regulate activation of nuclear factor κB and transcription of human immunodeficiency virus. Proc Natl Acad Sci 87:9943-9947.

Toshihiko, M., Ayoub, I.A., Sakakibara, Y., et al., 2000. Delayed treatment with nicotinamide (vitamin B_3) improves neurological outcome and reduces infarct volume after transient focal cerebral ischemia in Wistar rats. Stroke 31(7):1679-1685.

Treiman, D.M., 1993. Generalized convulsive status epilepticus in the adult. Epilepsia 34(S1):2.

Van Eijsden, P., Notenboom, R.G.E., Wu, O., De Grann, P.N.E.,et al., 2004. In vivo ^1H magnetic resonance spectroscopy, T_2-weighted and diffusion-weighted MRI during lithium-pilocarpine-induced status epilepticus in the rat. Brain Res 1030:11-18.

Wallenstein, M.C., Mauss, E.A., 1984. Effect of prostaglandin synthetase inhibitors on experimentally induced convulsions in rats. Pharmacology 29:85-93.

Wallenstein, M.C., 1985a. Differential effect of prostaglandin synthetase inhibitor pretreatment on pentylenetetrazol-induced seizures in rat. Arch Int Pharmacodyn 275:93-104.

Wallenstein, M.C., 1985b. Differential effects of prostaglandin synthetase inhibitors on EEG in rat. Eur J Pharmacol 111:201-209.

Wallenstein, M.C., 1987. Attenuation of penicillin models of epilepsy by nonsteroidal anti-inflammatory drugs. Exp Neurol. 98:152-160.

Wallenstein, M.C., 1991. Attenuation of epileptogenesis by nonsteroidal anti-inflammatory drugs in the rat. Neuropharm 30(6):657-663.

Wallis, R.A., Panizzon, K.L., Henry, D., et al., 1993.Neuroprotection against nitric oxide injury with inhibitors of ADP-ribosylation. NeuroReport 5:245-248.

Watson, G.B. and Lanthorn, T.H., 1990. Pharmacological characteristics of cyclic homologues of glycine at the N-methyl-D-aspartate receptor-associated glycine site. Neuropharm 29(8): 727-730.

Walton, N.Y., Treiman, D.M., 1991. Motor and electroencephalographic response of refractory experimental status epilepticus in rats to treatment with MK-801, diazepam or MK-801 plus diazepam. Brain Res 553:97-104.

Yang, Y., Li, Q., Shuaib, A., 2000. Neuroprotection by 2-h postischemia administration of two free radical scavengers, α-phenyl-n-tert-butyl-nitrone (PBN) and N-tert-butyl-(2-sulfophenyl)-nitrone (S-PBN), in rats subjected to focal embolic cerebral ischemia. Exp Neurol 163: 39-45.

Yue, T., Gu, J., Lysko, P.G., et al., 1992. Neuroprotective effects of phenyl-t-butyl-nitrone in gerbil global brain ischemia and in cultured rat cerebellar neurons. Brain Res. 574: 193-197.

Zhang, D.X., Bertram, E.H., 2002. Midline thalamic region: widespread excitatory input to the entorhinal cortex and amygdala. J Neurosci 22(8):3277-3284.

APPENDIX

Appendix Item 1:
Steven L. Peterson, Rebecca S. Purvis and James W. Griffith **Comparison of Neuroprotective Effects Induced by α-phenyl-N-*tert*-butyl nitrone (PBN) and N-*tert*-butyl-α-(2 sulfophenyl) nitrone (S-PBN) in Lithium-Pilocarpine Status Epilepticus. NeuroToxicology, In Press.**

Appendix Item 2:
Peterson, S.L., Purvis, R.S. and Griffith, J.W. **Differential Neuroprotective Effects of the NMDA Receptor-Associated Glycine Site Partial Agonists 1-Aminocyclopropanecarboxylic Acid (ACPC) and D-Cycloserine in Lithium-Pilocarpine Status Epilepticus. NeuroToxicology 25:835-847, 2004.**

Appendix Item 3:
Peterson, S.L., Purvis, R.S. and Griffith, J.W. **Propofol Neuroprotection in Cholinergic Status Epilepticus. Bioscience 2004 Abstracts.**

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Comparison of Neuroprotective Effects Induced by a-Phenyl-N-tert-butyl nitrone (PBN) and N-tert-Butyl-a-(2 sulfophenyl) nitrone (S-PBN) in Lithium-Pilocarpine Status Epilepticus

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Abstract

The status epilepticus (SE) induced in rats by lithium-pilocarpine (Li-pilo) shares many common features with soman-induced SE including extensive limbic neuropathology. Reactive oxygen species are hypothesized to play a role in the SE induced neuropathology and we propose that the free radical scavengers a-phenyl-N-tert-butyl nitrone (PBN) and N-tert-butyl-a-(2 sulfophenyl) nitrone (S-PBN) may be neuroprotective. PBN or S-PBN were administered either immediately following pilocarpine (exposure treatment) or 5 min after the onset of SE as determined by ECoG activity. SE was allowed to continue for 3 h before termination with propofol. The rats were sacrified 24 h following pilocarpine administration. S-PBN induced minor effects to reduce SE duration and improve neurological deficit 24 h following pilocarpine administration. One hundred and fifty milligrams per kilograms PBN administered 5 min after SE onset produced significant neuroprotection in the parietal, occipital, perirhinal and piriform cortices as well as the lateral amygdala. One hundred and fifty milligrams per kilograms S-PBN was neuroprotective only in the occipital and perirhinal cortex while 300 mg/kg S-PBN exacerbated cortical neuropathology. S-PBN administered 5 min after SE onset exacerbated neuropathology in thalamic regions. In contrast, PBN and S-PBN administered as exposure treatment exacerbated neuropathology in thalamic and CA3 regions. The differential neuroprotective effects of PBN and S-PBN may be the result of the poor brain penetration by S-PBN. The results suggest that free radical scavenger activity is neuroprotective in cortical regions during cholinergic convulsions. Regional variations in drug-induced neuroprotectant activity in Li-pilo SE are common and suggest multiple mechanisms of neuropathology.

Keywords: a-Phenyl-N-tert-butyl nitrone; N-tert-Butyl-a-(2 sulfophenyl) nitrone; Neuroprotection; Lithium; Pilocarpine; Status epilepticus

INTRODUCTION

Lithium administration in rats followed 20-24 h later by pilocarpine induces a continuous status epilepticus (SE) of several hours duration. Lithium-pilocarpine (Li-pilo) SE induces a characteristic pattern of limbic neuropathology that is particularly severe in midline thalamus regions, hippocampus, piriform cortex, perirhinal cortex and amygdala (Clifford et al., 1987). Following a latent period of several weeks the animals develop spontaneous recurrent seizures (Andre et al., 2001; Leite et al., 2002; Loscher, 2002). Because

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of the pharmacological, behavioral and pathological similarities, Li-pilo SE is considered a model of human temporal lobe epilepsy (Leite et al., 2002; Loscher, 2002) and has been tested as such (Andre et al., 2001, 2003; Rigoulot et al., 2003).

Li-pilo convulsions may serve as a model of the effects induced by the warfare nerve agent soman. Both Li-pilo (Jope et al., 1986) and soman-induced SE (Shih et al., 1997; McDonough and Shih, 1997) have an initial muscarinic phase that is followed by a secondary glutamatergic phase (Ormandy et al., 1989; McDonough and Shih, 1997). Both produce a similar pattern of neuropathology that is particularly severe in the limbic system (Clifford et al., 1987; McDonough et al., 1998). Li-pilo (Jope et al., 1986) and soman (Shih et al., 1997; McDonough and Shih, 1997) produce convulsions that are relatively refractory to drug therapy. Because such convulsions are resistant to anticonvulsant activity, we propose that neuroprotectants that reduce neuronal damage even during ongoing seizure activity would be of value.

α-Phenyl-N-tert-butylnitrone (PBN) is neuroprotectant in models of neuronal injury. PBN is neuroprotective in brain ischemia models in gerbils (Yue et al., 1992; Oliver et al., 1990) and in rats (Cao and Phillis, 1994; Li et al., 2001), even when administered as late as 12 h after the ischemic episode (Cao and Phillis, 1994). PBN has also been shown to be neuroprotective in experimental models of epilepsy. PBN (200 mg/kg) administered 30 min before kainic acid had no effect on the SE but induced a neuroprotective effect as measured by cytochrome C oxidase activity and energy metabolism (Milatovic et al., 2001). PBN (100 mg/kg) was neuroprotective in flurothyl-induced SE (He et al., 1997) and PBN (30 mg/kg) reduced hydroxyl radical formation in seizures induced by a pentylenetetrazol kindling paradigm (Rauca et al., 2004). PBN (two doses of 100 mg/kg) induced neuroprotection in the hippocampus of postictal day 25 (P25) rats subjected to Li-pilo SE (Rejchrtova et al., 2004).

N-tert-Butyl-α-(2-sulfophenyl) nitrone (S-PBN) is a sulfonated derivative of PBN that is significantly more water-soluble. The major difference is pharmacokinetic in that PBN has a plasma half-life of 3 h and readily penetrates the blood-brain barrier (BBB) (Chen et al., 1990) while S-PBN has a plasma half-life of 9 min and poor BBB penetration (Marklund et al., 2001a; Yang et al., 2000). Despite these reports systemically administered S-PBN induces significant CNS neuroprotection when tested using in vivo models of neural injury. For example, systemically administered S-PBN decreased central lesions induced by numerous excitotoxins (Schulz et al., 1995a). Systemic S-PBN reduced hypoxic lesion volume when tested as late as 6 h following the ischemic episode (Schulz et al., 1995b). Intraperitoneal PBN or S-PBN were equally effective in reducing infarct volume following embolic stroke in rats (Yang et al., 2000). Intravenous PBN or S-PBN were equally effective neuroprotectants in traumatic brain injury (TBI) models in rats (Marklund et al., 2001a,b, 2002). In two of the TBI studies S-PBN induced significantly greater neuroprotective effects than PBN (Marklund et al., 2001a, 2002). In spite of reportedly unfavorable pharmacokinetic properties, S-PBN induces significant neuroprotection in a wide variety of experimental models of excitotoxicity.

The purpose of this study was to test the relative activities of S-PBN and PBN as neuroprotectants in Li-pilo-induced SE as an experimental model of nerve agent exposure. As spin-trapping agents it was hypothesized that S-PBN and PBN will reduce SE-induced neuropathology mediated by reactive oxygen species.

METHODS

Animals

Male, Sprague-Dawley rats obtained from Harlan (Indianapolis, IN) and weighing 290–325 g at the time of seizure test were used for these experiments. The animals were maintained in a climate-controlled vivarium at 21 8C on a 12 h light/12 h dark cycle with food and water available ad libitum. All animal care and use conformed to the policies of the University of New Mexico Health Sciences Center.

Intracranial Implants

Rats were anesthetized with equithesin (a mixture of chloral hydrate, pentobarbital, magnesium sulfate, ethanol, propylene glycol and water) for the surgical placement of the electrocorticogram (ECoG) recording electrodes. Stainless steel ECoG recording screws were placed bilaterally in the skull 3 mm lateral to midline and equidistant between bregma and lambda. The screws were attached to connector pins by insulated wire. A third screw assembly was placed over the frontal sinus as a reference electrode and additional screws were set in the skull to serve as anchors. All

connector pins were inserted into a McIntyre connector (Ginder Scientific, Ottawa, ON). Screws, wires and connectors were secured in place with dental acrylic cement and the incision site closed with surgical staples. Postoperative antibiotics (25,000 IU Durapen) and analgesics (0.02 mg/kg buprenorphine) were administered. Animals were allowed 7–10 days recovery before seizure testing.

Seizure Induction and ECoG Recording

The rats were administered s.c. 3 mmol/kg lithium chloride (Sigma, St. Louis, MO) dissolved in normal saline the day prior to the seizure induction. Lithium administration always preceded the pilocarpine administration by 20–24 h. Twenty-four hours following pilocarpine the animals were placed in a seizure observation cage and connected by way of the implanted McIntyre connector to a Grass Model 8 electroencephalograph for ECoG recording. Pilocarpine 25 mg/kg (Sigma) dissolved in normal saline was administered s.c. following 10 min of baseline ECoG recording. ECoG activity was recorded continuously throughout the experiment and SE was defined as the occurrence of continuous high amplitude ECoG spiking (Ormandy et al., 1989).

S-PBN and PBN Testing

S-PBN (Sigma) and PBN (Sigma) were dissolved in saline and administered by i.p. injection in a volume of 4 ml/kg to assure adequate absorption (White et al., 1995). Being highly water soluble, S-PBN was tested in 150 and 300 mg/kg doses. With limited solubility PBN was tested only in the 150 mg/kg dose so that the 4 ml/kg injection volume was maintained. For the exposure treatment the test drugs were administered immediately following the pilocarpine administration. For the 5 min SE group the test drugs were administered 5 min after the onset of SE as determined by continuous high amplitude ECoG spiking.

Propofol Administration

Ongoing SE was terminated by propofol administered, i.p. as the commercially available injectable emulsion Propofo (Baxter Healthcare, New Providence, NJ). A 55 mg/kg dose of propofol was administered following 3 h of SE as defined by ECoG activity. Preliminary studies determined that this treatment terminated the Li-pilo SE and increased 24 h survival to 100%.

Spontaneous Activity Assessment

Spontaneous locomotor activity was used to measure the seizure-induced neurological deficit. The computer controlled Rodent Activity Analyser (Omnitech Electronics) system included activity monitor cages (40.5 cm □ 40.5 cm □ 20 cm) with two sets of 16 photocells located at right angles to each other to record horizontal activity. The activity cages were located in light and sound attenuated chambers. The spontaneous locomotor activity was evaluated during two 10 min test periods. The preseizure test occurred 24 h prior to pilocarpine and just before the lithium administration. The postseizure test occurred 24 h after pilocarpine administration and just prior to brain perfusion-fixation. The parameters measured were distance traveled (DT) and resting time (RT). Activity on the postseizure test was expressed as a percent of the preseizure test activity.

Histological Preparation and Digital Imaging

Animals were sacrificed 24 h following pilocarpine administration as that is a period after which extensive SE-induced neuropathology is observed by hematoxylin and eosin (H&E) staining techniques in Li-pilo (Clifford et al., 1987; Fujikawa et al., 1999) and soman-induced convulsions in rats (McDonough et al., 1998). All animals were anesthetized with equithesin when sacrificed by intraaortic perfusion-fixation. The animals were initially perfused with heparinized phosphate buffered saline (PBS) (12.5 IU/ml, Sigma) followed by 10% formalin PBS (VWR Scientific Products). Brains were removed 4–6 days following perfusion and immersed in 10% formalin for a minimum of 24 h of postfixation. The brains were subsequently paraffin embedded and sectioned into 5 mm sections by a rotary microtome (Microm International). Brain sections were mounted on glass slides and stained with H&E.

Tissue sections were taken every 125 mm through the brain tissue 0.8–4.8 mm posterior to bregma. This specific brain region was chosen for analysis because it contains a majority of the brain nuclei that exhibit the greatest degree of damage from soman (McDonough et al., 1998) and Li-pilo convulsions (Clifford et al., 1987; Fujikawa et al., 1999). A scale of lesion severity developed for assessing soman toxicity (McDonough et al., 1989) was used to score the neuronal damage. The scale was as follows: 0, none; 1, minimal = <5% necrotic or malacic tissue; 2, mild = 6–15% necrotic or malacic tissue; 3, moderate = 16–40% necrotic or

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malacic tissue; 4, severe = > 40% necrotic or malacic tissue. All sections were rated by a single observer (JG) in a blinded fashion. The mean damage score from a given region across all tissue sections was used as the neuronal damage score for statistical tests (McDonough et al., 1989).

Data and Statistical Analysis

Comparisons of the histopathological rating score parameters were performed using Kruskal–Wallis H-test for nonparametric statistical analysis. Statistical comparison of latency to SE onset, SE duration and spontaneous activity between groups was determined by analysis of variance (ANOVA) followed by Newman–Kuels post hoc test when a significant difference was determined by ANOVA. Values of $P < 0.05$ were considered significant for all statistical tests.

RESULTS

Li-pilo SE

A total of 132 rats completed the experimental paradigm and are included in the data analysis. Pilocarpine induced a SE characterized by continuous high amplitude ECoG spiking (Ormandy et al., 1989) as shown in Fig. 1. The average latency to SE onset in vehicle control animals was 33 min (Table 1). Prior to SE onset, isolated limbic seizures were observed (Fig. 1). Twenty-four hours following pilocarpine epileptiform ECoG spiking was still observed just prior to sacrifice and perfusion-fixation (Fig. 1).

Neither S-PBN nor PBN had a significant effect on the latency to SE onset or the duration of SE afterdischarge when administered as exposure treatment (Table 1). When administered 5 min following SE onset neither S-PBN nor PBN induced any significant changes in SE duration compared to control (Table 1). However, when administered 5 min following SE onset, both S-PBN doses produced average SE durations that were significantly less than that of the 150 mg/kg PBN dose (Table 1).

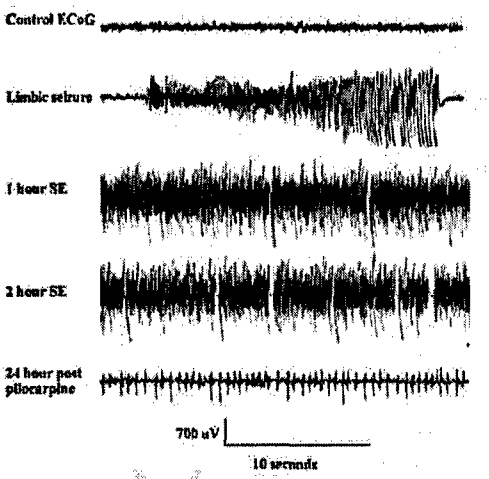

Fig. 1. Representative samples of ECoG tracings taken from Li-pilo-induced seizures. Initial limbic seizures similar in appearance to kindled amygdala seizures appeared first. This was followed by continuous, high amplitude spiking which defined SE. Intermittent epileptiform ECoG spiking was observed 24 h later. All of the tracings shown here are from a single rat treated with 150 mg/kg PBN 5 min following SE onset.

S-PBN and PBN Effect on Neurological Deficit

Neither S-PBN nor PBN administered as exposure treatment had any significant effect on Li-pilo SE-induced neurological deficit as determined by spontaneous behavior. The average distance traveled in the postseizure trial was 25–45% of the preseizure activity in all exposure treatment groups (Table 2). The average resting time in the postseizure trial was 179–224% the

Table 1
Effect of S-PBN and PBN on seizure activity

	Exposure treatment			
	Control (n = 8)	150 S-PBN (n = 9)	300 S-PBN (n = 7)	150 PBN (n = 9)
Latency to SE (min)	33.0 ± 1.6	35.6 ± 1.9	33.9 ± 3.3	36.7 ± 3.7
Duration of SE (min)	139.6 ± 4.3	121.3 ± 5.2	119.0 ± 8.3	127.4 ± 3.6
	5 min SE			
	Control (n = 8)	150 S-PBN (n = 8)	300 S-PBN (n = 9)	150 PBN (n = 8)
Latency to SE (min)	n/a	n/a	n/a	n/a
Duration of SE (min)	125.0 ± 2.8	117.5 ± 6.7[a]	119.2 ± 3.5[a]	135.6 ± 3.2

[a] Significantly different from 150 mg/kg PBN.

Table 2
Effect of S-PBN and PBN on spontaneous behavior

	Exposure treatment			
	Control (n = 8)	150 S-PBN (n = 9)	300 S-PBN (n = 7)	150 PBN (n = 9)
Distance traveled	38.0 □ 14.6	37.6 □ 13.5	44.9 □ 18.6	24.9 □ 7.6
Resting time	186.9 □ 24.8	203.0 □ 39.2	179.0 □ 31.2	223.7 □ 27.2
	5 min SE			
	Control (n = 8)	150 S-PBN (n = 8)	300 S-PBN (n = 9)	150 PBN (n = 8)
Distance traveled	23.3 □ 9.1	57.3 □ 17.1	86.3 □ 18.9[a]	35.3 □ 10.6
Resting time	228.3 □ 30.3	167.4 □ 33.4	146.9 □ 37.5	182.6 □ 30.2

[a] Significantly different from control values are a percent of pre SE control activity.

preseizure time in all exposure treatment groups (Table 2). Although the results were similar in the 5 min following SE onset treatment groups (Table 2), the 300 mg/kg S-PBN treatment group demonstrated significantly greater distance traveled as compared to control.

S-PBN and PBN Effect on Neuropathology

The brain structures evaluated in the vehicle control groups demonstrated mean neuronal damage scores of 1.3–4.0 corresponding from approximately 2–3% to greater than 40% necrotic or malacic tissue. This

Table 3
Effects of S-PBN and PBN on neural damage

Brain region	Exposure treatment			
	Control n = 8	150 S-PBN n = 8	300 S-PBN n = 7	150 PBN n = 9
Parietal cortex (Par1)	2.61	2.62	2.87	1.52[a]
Occipital cortex (Oc)	2.69	2.58	2.73	1.81[a]
Perirhinal cortex (PRh)	3.76	3.26	3.52	3.51
Piriform cortex (Pir)	4.00	4.00	3.79	4.00
Cortical amygdala (Co)	3.93	3.81	3.62[a]	4.00
Medial amygdala (BM)	3.16	3.42	3.19	3.79[a,b]
Lateral amygdala (La)	3.58	3.75	3.74	3.93
Mediodorsal thalamus (MD)	2.14	3.71[a,b]	3.41[a,b]	3.98[a,b]
Lateraldorsal thalamus (LD)	2.11	3.75[a,b]	3.58[a,b]	3.99[a,b]
Pretectal nucleus (APTD)	2.06	3.28[a,b]	3.08[a,b]	3.96[a,b]
CA3	2.49	3.47[a,b]	3.36[a,b]	3.59[a,b]
CA1	1.33	1.61	1.97	2.12
Amygdalopiri form (Apir)	4.00	3.89	4.00	4.00
Brain region	5 min SE			
	Control n = 8	150 S-PBN n = 8	300 S-PBN n = 9	150 PBN n = 8
Parietal cortex (Par1)	3.05	2.69	3.92[a,b]	1.57[a]
Occipital cortex (Oc)	3.00	2.59[a]	3.49[a,b]	1.63[a]
Perirhinal cortex (PRh)	3.77	3.07[a]	3.91	2.79[a]
Piriform cortex (Pir)	3.97	3.99	4.00	3.43[a]
Cortical amygdala (Co)	4.00	3.99	4.00	3.46[a]
Medial amygdala (BM)	3.62	3.51	3.64	3.17
Lateral amygdala (La)	3.88	3.81	4.00	3.24[a]
Mediodorsal thalamus (MD)	3.11	3.53[a,b]	3.69[a,b]	3.47
Lateraldorsal thalamus (LD)	3.15	3.73[a,b]	3.78[a,b]	3.47
Pretectal nucleus (APTD)	2.84	2.89	2.90	3.42[a,b]
CA3	2.83	3.45[a,b]	3.54[a,b]	3.50[a,b]
CA1	1.52	1.76	1.54	2.28
Amygdalopiri form (Apir)	4.00	4.00	4.00	3.49

[a] Indicates statistically significant difference from control group as determined by Kruskal-Wallis H-test.
[b] Indicates that neural damage was enhanced by S-PBN or PBN treatment.

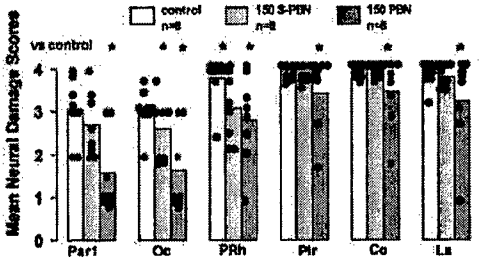

Fig. 2. Graphic presentation of the neuroprotection induced by 150 mg/kg S-PBN or 150 mg/kg PBN administered 5 min following the onset of SE. Both individual neuropathology scores and the group average are depicted. PBN neuroprotection was most prevalent in the cortical regions. S-PBN induced significant effects only in occipital and perirhinal cortex. Note that 300 mg/kg S-PBN induced little additional neuroprotection in any region (Table 3). Asterisk (*) indicates significant difference from the corresponding Li-pilo control group as determined by Kruskal–Wallis H-test. Abbreviations are as indicated in Table 1.

indicates that the Li-pilo model of soman toxicity induces a quantifiable degree of neuropathology in the expected brain regions (Clifford et al., 1987; Fujikawa et al., 1999; McDonough et al., 1989, 1998).

S-PBN induced little neuroprotection in Li-pilo-induced SE. Three hundred milligrams per kilograms S-PBN exposure treatment reduced neuropathology rating scores in the cortical amygdala (Table 3). One hundred and fifty milligrams per kilograms S-PBN administered 5 min following SE onset induced significant neuroprotection in the occipital and perirhinal cortices (Table 3). In contrast, both S-PBN doses at either administration time induced significant increases in neuropathology in the mediodorsal thalamus, lateraldorsal thalamus and CA3 regions (Table 3). S-PBN exposure treatment also increased neuronal damage in the pretectal nucleus. Three hundred milligrams per kilograms S-PBN administered following 5 min of SE increased neuropathology in the parietal and occipital cortex.

PBN induced both neuroprotection and exacerbated the neuronal damage in a region specific manner. One hundred and fifty milligrams per kilograms PBN administered 5 min following SE onset significantly reduced the neuronal lesion scores in the parietal, occipital, perirhinal and piriform cortices as well as the cortical and lateral amygdala regions (Table 3). These results are also presented graphically in Fig. 2. The neuroprotection was most obvious in the temporal lobe regions as the macroscopic lesions induced by the Li-pilo SE were reduced by PBN treatment (Fig. 3). At the microscopic level, the widespread pyknosis of nuclei, vacuolation and malacia in the cortical regions was reversed by the 150 mg/kg PBN treatment administered 5 min following SE onset (Fig. 4). One hundred and fifty milligrams per kilograms PBN exposure treatment also reduced neuronal damage in the parietal cortex and occipital cortex (Table 3).

PBN and S-PBN both exacerbated the neuropathology in the mediodorsal and lateraldorsal thalamus, pretectal nucleus and CA3 region (Table 3). PBN

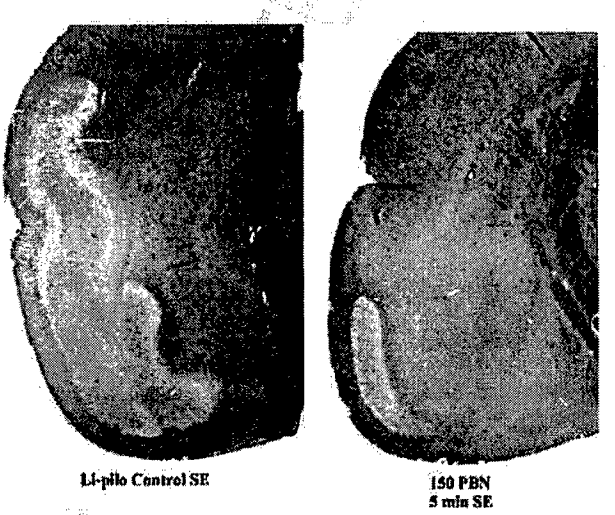

Fig. 3. H&E stained sections of the rat temporal lobe showing the macroscopic lesions resulting from Li-pilo-induced SE and the neuroprotection induced by 150 mg/kg PBN administered 5 min after SE onset. Scale bar equals 1 mm.

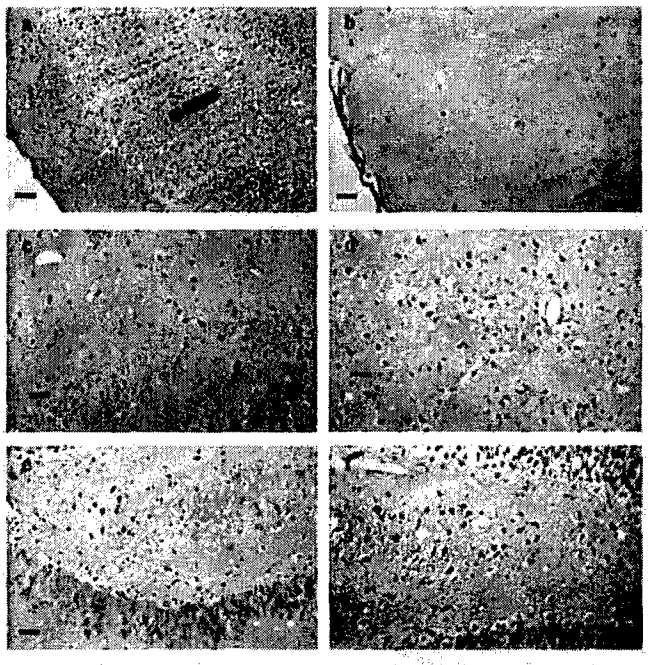

Fig. 4. Representative high magnification H&E sections of rat brains showing the severity of Li-pilo-induced neuropathology and examples of the variable effects of PBN. (a) Parietal cortex from a vehicle control rat brain showing greater than 40% necrosis. Notice the widespread pyknosis of nuclei, vacuolation and malacia. (b) Parietal cortex from a PBN treated rat showing 1–5% necrosis. Notice the small number of pyknotic nuclei. (c) Section of mediodorsal thalamus from vehicle control brain showing 6–15% necrosis. Notice the pyknotic nuclei and mild vacuolation. (d) Mediodorsal thalamus from PBN treated rat brain showing greater than 40% necrosis. Notice the wide spread pyknosis of nuclei, angulated dark cells, vacuolation and malacia. (e) Section of CA3 region of hippocampus from a vehicle control rat brain showing 6–15% necrosis. Notice the pyknotic nuclei and mild vacuolation. (f) CA3 region of PBN treated rat showing greater than 40% necrosis. Notice the pyknosis, vacuolation and malacia within CA3 region cells. Calibration bars = 100 m

induced wide spread pyknosis of nuclei, angulated dark cells, vacuolation and malacia in the mediodorsal thalamus and the CA3 region (Fig. 4).

DISCUSSION

PBN induced significant neuroprotection in cortical and amygdala regions when administered 5 min following SE onset. This collaborates previous reports of PBN neuroprotection in experimental models of neuronal injury (Yue et al., 1992; Oliver et al., 1990; Cao and Phillis, 1994; Li et al., 2001) and experimental models of epilepsy (He et al., 1997; Milatovic et al., 2001; Rauca et al., 2004; Rejchrtova et al., 2004). The neuroprotection in Li-pilo SE resulted from a single administration of PBN whereas the neuroprotection induced by pregabalin (Andre et al., 2003), vigabatrin (Andre et al., 2001) and caffeine (Rigoulot et al., 2003) required chronic administration. Given that repeated PBN administration paradigms have been successful in other models of brain injury (Cao and Phillis, 1994; Yang et al., 2000; Marklund et al., 2001b; Li et al., 2001; Rejchrtova et al., 2004) we expect that a repeated PBN administration paradigm would result in even greater neuroprotection in SE.

The effects of PBN and S-PBN were not equivalent. S-PBN administered 5 min following SE onset reduced SE duration as compared to PBN (Table 1) and the 300 mg/kg S-PBN dose reduced the SE-induced neurological deficit as measured by distance traveled in the spontaneous behavior evaluation (Table 2). However, these apparent beneficial effects of S-PBN were not paralleled in the neuropathology evaluation as PBN produced significant reductions in cortical and amygdala lesions while S-PBN did not. Even the 300 mg/kg S-PBN dose, a dose 27% greater than the 150 mg/kg PBN dose on a molar basis, induced little neuroprotec-

tion compared to 150 mg/kg PBN. This may be expected given that PBN has a plasma half-life of 3 h and readily penetrates the BBB (Chen et al., 1990) while S-PBN has a plasma half-life of 9 min and poor BBB penetration (Marklund et al., 2001a; Yang et al., 2000). However, these results contrast with reports of equivalent neuroprotection in various in vivo models of stroke and traumatic brain injury (Schulz et al., 1995a,b; Yang et al., 2000; Marklund et al., 2001a,b, 2000). Due to the poor brain penetration of S-PBN, the comparable effects of PBN and S-PBN in ischemic stroke and traumatic brain injury have been hypothesized to result from an action at the blood-endothelial interface where the compounds attenuate microvascular reactive oxygen species production (Marklund et al., 2001a,b). The results of the present study suggest PBN acts beyond the BBB at sites within cortical regions where S-PBN does not penetrate. Although SE is known to disrupt the BBB, there is little or no increase in BBB permeability in the piriform or entorhinal cortex during Li-pilo SE (Leroy et al., 2003).

PBN administered 5 min following SE onset provided more neuroprotection than the exposure treatment. Intraperitoneal PBN administration produces peak brain PBN levels at 30 min (Chen et al., 1990). In the present experiment the average onset to SE was 35 min. This suggests that the brain levels of PBN were decreasing in the exposure treatment group just as the SE was beginning. Repeated PBN administration paradigms have been successful in other models of nerve injury (Marklund et al., 2001b; Li et al., 2001; Rejchrtova et al., 2004). Interestingly, PBN administration at 12 or 24 h intervals over several days is neuroprotective following ischemic stroke (Cao and Phillis, 1994; Yang et al., 2000). This suggests that repeated PBN treatment may provide additional neuroprotection during Li-pilo SE.

SE-induced NMDA receptor activation has been proposed to increase the production of ROS that mediate the observed neuropathology and pathophysiology (Lafon-Cazal et al., 1993; Bruce and Baudry, 1995; Rong et al., 1999). There is indirect evidence of ROS production in various models of SE (Rong et al., 1999; Bruce and Baudry, 1995) including Li-pilo (Peterson et al., 2002). Antioxidants and free radical spin trap agents that inactivate ROS reduce the neuropathology associated with SE (Rong et al., 1999; Schulz et al., 1995a). PBN and S-PBN are spin-trapping agents that react with ROS to form a more stable nitroxide free radical (Chen et al., 1990; Yang et al., 2000). When tested directly in brain injury models, PBN and S-PBN have been shown to produce significant reductions in hydroxyl radical accumulation (Sen et al., 1994; Ferger et al., 1998; Lancelot et al., 1998; Marklund et al., 2001a; Rauca et al., 2004). Given that S-PBN and PBN do not antagonize glutamate receptor activity in physiological preparations (Schulz et al., 1995a; Zivin et al., 1999), we propose free radical scavenging activity by the BBB permeable PBN induced the neuroprotection observed in Li-pilo SE.

Neuroprotectants administered during Li-pilo SE induce region specific effects on seizure-induced neuropathology. Vigabatrin induced neuroprotection in hippocampal regions but exacerbated neuropathology in the entorhinal cortex (Andre et al., 2001). Caffeine also produced hippocampal neuroprotection but exacerbated piriform cortex neuropathology (Rigoulot et al., 2003). Pregabalin produced no hippocampal neuroprotection but significantly reduced piriform and entorhinal neuropathology (Andre et al., 2003). Using the same paradigm as the current experiment, 1-aminocycloprop anecarboxylic acid (ACPC) was neuroprotectant in parietal, occipital, piriform and perirhinal cortices but enhanced neuropathology in thalamic regions (Peterson et al., 2004). In the present study, the observed PBN neuroprotection in the piriform and entorhinal cortex may represent antiepileptogenic activity as neuroprotection in these areas has been shown to delay the onset of spontaneous recurrent seizures (Andre et al., 2003). The exacerbation of thalamic neuropathology by PBN and S-PBN is of interest as the mediodorsal nucleus plays a critical role in the development of acute limbic SE (Zhang and Bertram, 2002) and is the region that most consistently shows neuronal damage in limbic SE (Bertram and Scott, 2000). The extensive increase in BBB permeability in the thalamus during Li-pilo SE (Leroy et al., 2003; Van Eijsden et al., 2004) would explain why both S-PBN and PBN induced an effect in this region. The exacerbated neuropathology is likely a drug-seizure interaction as PBN administration alone does not induce neuropathology (Rejchrtova et al., 2004). Although the exacerbated neuropathology may result from free radical scavenger activity, PBN does have other activity including acetylcholinesterase inhibition (Zivin et al., 1999), calcium channel blockade (Anderson et al., 1993) and suppression of inducible nitric oxide synthase (Hensley et al., 1997) which may contribute to the thalamic pathology.

The PBN-induced reduction in neuronal lesion scores was not correlated with improved spontaneous locomotor activity when assessed 24 h following Li-pilo SE. This is similar to our previous finding with

ACPC (Peterson et al., 2004). Reports from other studies using cholinergic convulsions indicates that behavior 24 h following SE induction is normal only in the absence of any neurodegeneration (Filliat et al., 1999; Hort et al., 1999; Balakrishnan et al., 2001). The central and peripheral effects of ongoing SE are probably sufficient to disrupt all behavior in the first few days following SE. This suggests 24 h post SE behavioral assessment would only be relevant in the case of anti-epileptic treatments that induce an early, immediate termination of SE. Long-term cognitive and behavioral benefits of neuroprotectant treatments would be more appropriately assessed weeks to months following SE.

PBN has anticonvulsant and neuroprotectant activity in Li-pilo SE induced in postnatal day (P25) rats. Although Li-pilo SE induces neuropathology in P25 rats the convulsions in the young rats were characterized by a high incidence of wild running and generalized tonic-clonic seizures (Rejchrtova et al., 2004) whereas adult rats display immobility with staring and a mild clonus of the head and forelimbs. The differences in seizure type and underlying mechanisms may explain why PBN increased the latency to SE and decreased seizure severity in P25 (Rejchrtova et al., 2004) but not adult rats as shown in the present study. Similarly, PBN reduced neuropathology in septal CA1 and CA3 regions in P25 rats (Rejchrtova et al., 2004) but had no effect in CA1 and exacerbated neuropathology in CA3 in the present study. These differences may relate to delayed development of CA1 synaptic inhibition and the ongoing maturation of mossy fiber terminals in CA3 of P25 rats (Sankar et al., 1998). In addition, wild running and tonic-clonic convulsions involve brainstem mechanisms that are separate mechanism from the forebrain and limbic neurocircuitry that mediate cholinergic SE (Browning, 1987). The variable effects of vigabatrin (Andre et al., 2001), caffeine (Rigoulot et al., 2003) and pregabalin (Andre et al., 2003) on CA1 and CA3 neuropathology suggests multiple mechanisms in adult Li-pilo SE induced neuropathology as well.

In conclusion, PBN induced significant neuroprotection in cortical areas while S-PBN did not. PBN neuroprotection is hypothesized to result from free radical scavenging activity within the brain where S-PBN cannot penetrate the BBB. Both PBN and S-PBN exacerbated thalamic neuropathology possibly due to enhanced thalamic BBB permeability during Li-pilo SE. Regional variability in response to neuroprotectants is common in Li-pilo SE and suggests multiple mechanisms of neuropathology that are influenced by age of the animal.

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REFERENCES

Anderson DE, Yuan XJ, Teeng CM, Rubin JL, Rosen GM, Tod ML. Nitrone spin traps block calcium channels and induce pulmonary artery relaxation independent of free radicals. Biochem Biophys Res Commun 1990;193:878–85.

Andre A, Ferrandon A, Marescaux C, Nehlig A. Vigabatrin protects against hippocampal damage but is not antiepileptogenic in the lithium-pilocarpine model of temporal lobe epilepsy. Epilepsy Res 2001;47:99–117.

Andre A, Rigoulot AM, Koning E, Ferrandon A, Nehlig A. Long-term pregabalin treatment protects basal cortices and delays the occurrence of spontaneous seizures in the lithium-pilocarpine model in the rat. Epilepsia 2003;44(7):893–903.

Balakrishnan S, Nidhi G, Pandhi P. Effect of duration of pilocarpine-induced status epilepticus on subsequent cognitive function in rats. Methods Find Exp Clin Pharmacol 2001;23(2):85–7.

Bertram EH, Scott C. The pathological substrate of limbic epilepsy: neuronal loss in the medial dorsal thalamic nucleus as the consistent change. Epilepsia 2000;41(S6):S3–8.

Browning RA. Effects of lesions on seizures in experimental animals. In: Fromm GH, Faingold RA, Browning RA, Burnham WM, editors. Neurology and neurobiology, vol. 27. Epilepsy and the reticular formation: the role of the reticular core in convulsive seizures. New York: Alan Liss; 1987. 137–62.

Bruce AJ, Baudry M. Oxygen free radicals in the rat limbic structures after kainate-induced seizures. Free Radic Biol Med 1995;18:993–1002.

Cao X, Phillis JW. a-Phenyl-tert-butyl-nitrone reduces cortical infarct and edema in rats subjected to focal ischemia. Brain Res 1994;644:267–72.

Chen GM, Bray TM, Janzen EG, McDay PB. Excretion, metabolism and tissue distribution of a spin trapping agent, a-phenyl-N-tert-butyl-nitrone (PBN) in rats. Free Radic Res Commun 1990;9:317–23.

Clifford DB, Olney JW, Maniotis A, Collins RC, Zorumski CF. The functional anatomy and pathology of lithium-pilocarpine and high-dose pilocarpine seizures. Neuroscience 1987;23(3):953–68.

Fergar B, van Amsterdam C, Seyfried C, Kuschinsky K. Effects of a-phenyl-tert-butylnitrone and selegiline on hydroxyl free radicals in rat striatum produced by local application of glutamate. J Neurochem 1998;70:276–80.

Filliat P, Baubichon D, Burckhart M, Pernot-Marino I, Fequin A, Masqueliez C, et al. Memory impairment after soman intox-

583 ication in rat: correlation with central neuropathology. Improve-
584 ment with anticholinergic and antiglutamatergi c therapeutics.
585 NeuroToxicology 1999;20(4):535 –50.
586 Fujikawa DG, Shinmei SS, Cai B. Lithium-pilocarp ine-induced
587 status epilepticus produces necrotic neurons with internucleo-
588 somal DNA fragmentation in adult rats. Eur J Neurosci 1999;
589 11:1605 –14.
590 He QP, Smith ML, Li PA, Siesjo BK. Necrosis of the substantia
591 nigra, pars reticulata, in flurothyl-indu ced status epilepticus is
592 ameliorated by the spin trap alpha phenyl- N-tert-butyl nitrone.
593 Free Radic Biol Med 1997;22(5):917 –22.
594 Hensley K, Carney JM, Steward CA, Tabatabaie T, Pye Q, Floyd
595 RA. Nitrone-based free radical traps as neuroprotective agents
596 in cerebral ischemia and other pathologies. Int Rev Neurobiol
597 1997;40 :299 –317.
598 Hort J, Brozek G, Mares P, Langmeier M, Komarek V. Cog-
599 nitive functions after pilocarpine-indu ced status epilep-
600 ticus: changes during silent period precede appearance
601 of spontaneous recurrent seizures. Epilepsia 1999;40(9):
602 1177 –83.
603 Jope RS, Morrisett RA, Snead OC. Characterization of lithium
604 potentiat ion of pilocarpine-indu ced status epilepticus in rats.
605 Exp Neurol 1988;91:471 –80.
606 Lafon-Cazal M, Culcasi M, Gaven F, Pietri S, Bockaert J. Nitric
607 oxide, superoxide and peroxynitrite: putative mediators of
608 NMDA-induced cell death in cerebellar granule cells. Neuro-
609 pharmacolo gy 1993;32(11):1259 –66.
610 Lancelot E, Revaud M, Boulu RG, Plotkine M, Callebert J. A
611 microdialysis study investigating the mechanisms of hydroxyl
612 radical formation in rat striatum exposed to glutamate. Brain
613 Res 1998;809:294 –6.
614 Leite JP, Garcia-Cairasco N, Cavalheiro EA. New insights from the
615 use of pilocarpine and kainate models. Epilepsy Res
616 2002;50:93 –103.
617 Leroy C, Roch C, Koning E, Namer IJ, Nehlig A. In the lithium-
618 pilocarpine model of epilepsy, brain lesions are not linked to
619 changes in blood–brain barier permeability: an autoradio-
620 graphi c study in adult and developing rats. Exp Neurol
621 2003;182:3 61–72.
622 Li PA, He QP, Nakamura L, Csiszar K. Free radical spin trap a-
623 phenyl- N-tert-butyl-nitron e inhibits caspase-3 activation and
624 reduces brain damage following a severe forebrain ischemic
625 injury. Free Radic Biol Med 2001;31(10):1191 –7.
626 Loscher W. Animal models of epilepsy for the development
627 of antiepileptogeni c and disease-modifyin g drugs. A compar-
628 ison of the pharmacology of kindling and post-status epilep-
629 ticus models of temporal lobe epilepsy. Epilepsy Res 2002;
630 50:105 –23.
631 Marklund N, Lewander T, Clausen F, Hillered L. Effects of the
632 nitrone radical scavengers PBN and S-PBN on in vivo trapping
633 of reactive oxygen species after traumatic brain injury in rats. J
634 Cereb Blood Flow Metab 2001;21:1259 –67.
635 Marklund N, Clausen F, McIntosh TK, Hillered L. Free radical
636 scavenger posttreatment improves functional and morphologi-
637 cal outcome after fluid percussion injury in the rat. J Neuro-
638 trauma 2001;18(8):821 –32.
639 Marklund N, Sihver S, Langstrom B, Bergstrom M,
640 Hillered L. Effect of traumatic brain injury and nitrone radical
641 scavenger s on relative changes in regional cerebral blood flow
642 and glucose uptake in rats. J Neurotrauma 2002;19(10):
1139 –53.

644 McDonough JH, Jaax NK, Crowley RA, Mays MZ, Modrow HE.
645 Atropine and/or diazepam therapy protects against soman-
646 induced neural and cardiac pathology. Fundam Appl Toxicol
647 1989;13:2 56–76.
648 McDonough JH, Shih TM. Neuropharmacolog ical mechanisms of
649 nerve agent-induced seizure and neuropathology. Neurosci
650 Biobehav Rev 1997;21(5):559 –79.
651 McDonough JH, Clark TR, Slone TW, Zoeffel D, Brown K, Kim S,
652 et al. Neural lesions in the rat and their relationship to EEG delta
653 activity following seizures induced by the nerve agent soman.
654 Neurotoxic ology 1998;19(3):381 –92.
655 Milatovic D, Zivin M, Gupta RC, Dettbarn WD. Alterations in
656 cytochrome C oxidase activity and energy metabolites in
657 response to kainic acid-induced status epilepticus. Brain Res
658 2001;912:67 –78.
659 Oliver CN, Starke-Reed PE, Stadtman ER, Liu GJ, Carney JM,
660 Floyd RE. Oxidative damage to brain proteins, loss of glutamine
661 synthet ase activity, and production of free radicals during
662 ischemia/r eperfusion-induc ed injury to gerbil brain. Proc Natl
663 Acad Sci 1990;87:5144 –7.
664 Ormandy GC, Jope RS, Snead OC. Anticonvulsant actions of MK-
665 801 on the lithium-pilocarp ine model of status epilepticus in
666 rats. Exp Neurol 1989;105:172 –80.
667 Peterson SL, Morrow D, Liu S, Liu KJ. Hydroethidine detection of
668 superoxide production during the lithium-pilocarp ine model of
669 status epilepticus. Epilepsy Res 2002;49:226 –38.
670 Peterson SL, Purvis RS, Griffith JW. Differential neuroprotective
671 effects of the NMDA receptor-associa ted glycine site partial
672 agonists 1-aminocycloprop anecarboxylic acid (ACPC) and o-
673 cycloserine in lithium-pilocarp ine status epilepticus. NeuroTox-
674 icology 2004;25:835 –47.
675 Rauca C, Wiswedel I, Zerbe R, Kellhoff G, Krug M. The role of
676 superoxide dismutase and a-tocopherol in the development of
677 seizures and kindling induced by pentylenetetrazo l-influence of
678 the radical scavenger a-phenyl-N-tert-butyl nitrone. Brain Res
679 2004;1009:203 –12.
680 Rejchrtova J, Kubova H, Druga R, Mares P, Folbergrova J. Effects
681 of a free radical scavenger N-tert-butyl-alpha-phen ylnitrone
682 (PBN) on short-term recovery of immature rats after status
683 epilepticu s. Physiol Res 2004.
684 Rigoulot MA, Leroy C, Koning E, Ferrandon A, Nehlig A.
685 Prolonged low-dose caffeine exposure protects against hippo-
686 campal damage but not against the occurrence of epilepsy in the
687 lithium-piloc arpine model in the rat. Epilepsia 2003;44(4):
688 529–35.
689 Rong Y, Doctrow SR, Tocco G, Baudry M. EUK-134 a synthetic
690 superox ide dismutase and catalase mimetic, prevents oxidative
691 stress and attenuates kainate-induced neuropathology. Proc Natl
692 Acad Sci 1999;96:9897 –902.
693 Sankar R, Shin DH, Liu H, Mazarati A, Pereira de Vasconcelos A,
694 Wasterlain CG. Patterns of status epilepticus-indu ced neuronal
695 injury during development and long-term consequences. J
696 Neurosci 1998;18(2):8382 –93.
697 Sen S, Goldman H, Morehead M, Murphy S, Phillis JW. a -Phenyl-
698 tert-butyl-nitrone inhibits free radical release in brain concus-
699 sion. Free Radic Biol Med 1994;16(6):685 –91.
700 Schulz JB, Henshaw DR, Siwek D, Jenkins BG, Ferrante RJ,
701 Cipolloni PB, et al. Involvement of free radicals in excitotoxi-
702 city in vivo. J Neurochem 1995;64:2239 –47.
703 Schulz JB, Matthews RT, Jenkins BG, Brar P, Beal MF. Improved
therapeutic window for treatment of histotoxic hypoxia with a

free radical spin trap. J Cereb Blood Flow Metab 1995;15: 948–52.
Shih TM, McDonough JH, Koplovitz I. Evaluation of anticonvulsant drugs for soman-induced seizure activity. J Am Col Toxicology 1997;15(S2):43–60.
Van Eijsden P, Notenboom RGE, Wu O, de Graan PNE, van Nieuwenhuized O, Nicolay K, et al. In vivo ^{1}H magnetic resonance spectroscopy, T_2-weighted and diffusion-weighted MRI during lithium-pilocarpine-induced status epilepticus in the rat. Brain Res 2004;1030:11–8.
White HS, Woodhead JH, Franklin MR, Swinyard EA, Wolf HH. Experimental selection, quantification, and evaluation of antiepileptic drugs. In: Levy HH, Mattson RH, Meldrum BS, editors. Antiepileptic drugs. New York: Raven Press; 1995. 99–110.
Yang Y, Li Q, Shuaib A. Neuroprotection by 2-h postischemia administration of two free radical scavengers, α-phenyl-n-tert-butyl-nitrone (PBN) and N-tert-butyl-(2-sulfophenyl)-nitrone (S-PBN), in rats subjected focal embolic cerebral ischemia. Exp Neurol 2000;163:39–45.
Yue TL, Gu JL, Lysko PG, Cheng HY, Barone FC, Feuerstein G. Neuroprotective effects of phenyl-t-butyl-nitrone in gerbil global brain ischemia and in cultured rat cerebellar neurons. Brain Res 1992;574:193–7.
Zhang DX, Bertram EH. Midline thalamic region: widespread excitatory input to the entorhinal cortex and amygdala. J Neurosci 2002;22(8):3277–84.
Zivin M, Milatovic D, Dettbarn WD. Nitrone spin trapping compound N-tert-butyl-α-phenylnitrone prevents seizures induced by anticholinesterases. Brain Res 1999;850:63–72.

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Differential Neuroprotective Effects of the NMDA Receptor-Associated Glycine Site Partial Agonists 1-Aminocyclopropanecarboxylic Acid (ACPC) and D-Cycloserine in Lithium-Pilocarpine Status Epilepticus

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Abstract

The status epilepticus (SE) induced in rats by lithium-pilocarpine (Li-pilo) shares many common features with soman-induced SE including a glutamatergic phase that is inhibited by NMDA antagonists. The present study determined whether 1-aminocyclopropanecarboxylic acid (ACPC) or D-cycloserine (DCS), both partial agonists of the strychnine-insensitive glycine site on the NMDA receptor ionophore complex, exerted anticonvulsant or neuroprotectant activity in Li-pilo SE. ACPC or DCS were administered either immediately following pilocarpine (exposure treatment) or 5 min after the onset of SE as determined by ECoG activity. SE was allowed to proceed for 3 h before termination with propofol. The rats were sacrificed 24 h following pilocarpine administration. Neither drug had an effect on the latency to seizure onset or the duration of seizure activity. ACPC administered 5 min after SE onset produced significant neuroprotection in cortical regions, amygdala and CA1 of the hippocampus. In contrast, when administered as exposure treatment ACPC enhanced the neural damage in the thalamus and CA3 of the hippocampus suggesting the neuropathology in those regions is mediated by a different subset of NMDA receptors. DCS had no neuroprotectant activity in Li-pilo SE but exacerbated neuronal damage in the thalamus. Neither drug affected the cholinergic convulsions but both had differential effects on neural damage. This suggests that the SE-induced seizure activity and subsequent neuronal damage involve independent mechanisms.

Keywords: D-Cycloserine; 1-Aminocyclopropanecarboxylic acid; Neuroprotection; Lithium; Pilocarpine; Status epilepticus

INTRODUCTION

Rats treated with lithium chloride 20–24 h prior to pilocarpine administration develop status epilepticus (SE) of several hours duration (Clifford et al., 1987; Honchar et al., 1983). If allowed to proceed the SE produces significant neuropathology in the cortex, thalamus, hippocampus and amygdala regions (Clifford et al., 1987; Dubé et al., 2000, 2001; Honchar et al., 1983; Motte et al., 1998; Peredery et al., 2000). Following a latent period of several weeks the animals that survive the SE develop spontaneous recurrent limbic seizures as a result of the neuropathology (Dubé et al., 2000, 2001).

Lithium-pilocarpine (Li-pilo) convulsions model the cholinergic convulsions induced by the organophosphorus nerve agent soman. Both Li-pilo (Jobe et al., 1986; Morrisett et al., 1987) and soman-induced SE

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(McDonough and Shih, 1993, 1997) have an initial cholinergic phase that is inhibited by muscarinic antagonists. The SE induced by either Li-pilo (Ormandy et al., 1989; Walton and Treiman, 1991) or soman (Shih, 1990; McDonough and Shih, 1993, 1997; McLean et al., 1992) has a secondary glutamatergic phase that is inhibited by glutamate antagonists (McDonough and Shih, 1997; Solberg and Belkin, 1997). Both chemoconvulsants produce a similar pattern of neuropathology that is particularly severe in the limbic system (Clifford et al., 1987; McDonough et al., 1998; Motte et al., 1998; Peredery et al., 2000).

NMDA receptor antagonists are effective anticonvulsants in both soman (Shih, 1990; McDonough and Shih, 1993; McLean et al., 1992) and Li-pilo convulsions (Ormandy et al., 1989; Walton and Treiman, 1991). Unfortunately both competitive and noncompetitive NMDA antagonists typically induce undesirable psychotomimetic adverse effects (Rogawski, 1992). One potential mechanism for alternative treatment might involve the strychnine-insensitive glycine receptor which is an allosteric glycine recognition site located on the NMDA receptor/ionophore complex (Bonhaus et al., 1987; Bowery, 1987). Glycine acts at this site as a required co-agonist (Kleckner and Dingledine, 1988) to facilitate the opening of the NMDA receptor cation channel in a strychnine-insensitive manner (Johnson and Ascher, 1987). Antagonists of the strychnine-insensitive glycine site inhibit NMDA receptor activity (Kemp et al., 1988; Kleckner and Dingledine, 1989) but also induce unacceptable levels of psychotomimetic activity (Rogawski, 1992). However, partial agonists of the strychnine-insensitive glycine site may inhibit NMDA receptor activity without inducing psychotomimetic activity (Rogawski, 1992; Wlaź, 1998). As high efficacy agonists, partial agonists would have minimal psychotomimetic activity but would act as antagonists during periods of intense NMDA receptor activity such as that induced by cholinergic convulsions (McDonough and Shih, 1997; Nahum-Levy et al., 1999; Solberg and Belkin, 1997).

D-Cycloserine (DCS) is a partial agonist of the strychnine-insensitive glycine site with 40–70% the efficacy of glycine (Henderson et al., 1990; Hood et al., 1989; Karcz-Kubicha et al., 1997). DCS has anticonvulsant activity in maximal electroshock seizures (Peterson, 1992; Wlaź, 1998) and kindled amygdaloid seizures (Loscher et al., 1994) with minimal behavioral toxicity (Wlaź, 1998). DCS also induces significant reductions in the behavioral component of kainate seizures (Baran et al., 1994). Because of this anticonvulsant profile, DCS was deemed an ideal candidate to test the hypothesis of strychnine-insensitive glycine site partial agonists as anticonvulsants in Li-pilo SE.

1-Aminocyclopropanecarboxylic acid (ACPC) is also a high efficacy partial agonist of the strychnine-insensitive glycine receptor with 60–92% the efficacy of glycine (Karcz-Kubicha et al., 1997; Marvizon et al., 1989; Watson and Lanthorn, 1990). ACPC has anticonvulsant activity in NMDA-induced seizures in mice (Bisaga et al., 1993; Skolnick et al., 1989) and audiogenic seizures in genetically epilepsy-prone rats (Smith et al., 1993) with minimal behavioral toxicity (Skolnick et al., 1989; Smith et al., 1993). ACPC also possess neuroprotective activity in that it inhibits glutamate-induced cell damage in cerebellar granule cell cultures (Boje et al., 1993; Fossom et al., 1995), protects against ischemic cell damage in an experimental model of ischemic stoke (Fossom et al., 1995) and reduces neuronal damage in an experimental model of spinal cord injury (Long and Skolnick, 1994). Due to the anticonvulsant and neuroprotectant properties, ACPC was also tested for activity in the Li-pilo model of cholinergic convulsions.

METHODS

Animals

These experiments used male, Sprague–Dawley rats obtained from Harlan (Indianapolis, IN) and weighing 290–325 g at the time of seizure test. The animals were maintained in a climate-controlled vivarium at 21 °C on a 12-h light/12-h dark cycle with food and water available ad libitum. All animal care and use conformed to the policies of the University of New Mexico Health Sciences Center.

Intracranial Implants

Rats were anesthetized with equithesin (a mixture of chloral hydrate, pentobarbital, magnesium sulfate, ethanol, propylene glycol and water) for the surgical placement of the electrocorticogram (ECoG) recording electrodes. Stainless steel ECoG recording screws were placed bilaterally in the skull 3 mm lateral to midline and equidistant between bregma and lambda. The screws were attached to connector pins by insulated wire. A third screw assembly was placed over the frontal sinus as a reference electrode and additional screws were set in the skull to serve as anchors. All connector pins were inserted into a McIntyre connector (Ginder Scientific, Ottawa, ON). Screws, wires and

connectors were secured in place with dental acrylic cement and the incision site closed with surgical staples. Postoperative antibiotics (25,000 IU Durapen) and analgesics (0.02 mg/kg buprenorphine) were administered. Animals were allowed 7–10 days recovery before seizure testing.

Seizure Induction and ECoG Recording

The day prior to the seizure induction the rats were administered s.c. 3 mmol/kg lithium chloride (Sigma, St. Louis, MO, USA) dissolved in normal saline. The lithium administration always preceded the pilocarpine administration by 20–24 h. The following day the animals were placed in a seizure observation cage and connected to a Grass Model 8 electroencephalograph by way of the implanted McIntyre connector for recording of ECoG. Pilocarpine (Sigma) dissolved in normal saline was administered s.c. in a dose of 25 mg/kg following 10 min of baseline ECoG recording. ECoG activity was recorded continuously throughout the experiment. SE was defined as the occurrence of continuous high amplitude ECoG spiking (Ormandy et al., 1989).

ACPC and DCS Testing

ACPC (Sigma) and DCS (Sigma) were dissolved in saline and administered by i.p. injection in a volume of 4 ml/kg to assure adequate absorption (White et al., 1995). For the exposure treatment ACPC or DCS were administered immediately following the pilocarpine administration. For the 5 min SE group the test drugs were administered 5 min after the onset of SE as determined by continuous high amplitude ECoG spiking.

Propofol Administration

The ongoing SE was terminated by propofol administered i.p. as the commercially available injectable emulsion Propoflo (Baxter Healthcare, New Providence, NJ. USA). A 55 mg/kg dose of propofol was administered following 3 h of SE as defined by ECoG activity. Preliminary studies determined that this treatment terminated the Li-pilo SE and increased 24 h survival to 100%.

Spontaneous Activity Assessment

A computer controlled Rodent Activity Analyser (Omnitech Electronics) was used to determine spontaneous locomotor activity as a measure of seizure-induced neurological deficit. The system included activity monitor cages (40.5 cm × 40.5 cm × 20 cm) with two sets of 16 photocells located at right angles to each other to record horizontal activity. The activity cages were located in light and sound attenuated chambers. The spontaneous locomotor activity was determined over two 10 min test periods. The preseizure test occurred 24 h prior to pilocarpine and just before the lithium administration. The postseizure test occurred 24 h after pilocarpine administration and just prior to brain perfusion-fixation. The parameters measured were distance traveled (DT) and resting time (RT). Activity on the postseizure test was expressed as a percent of the preseizure test activity.

Histological Preparation and Digital Imaging

Animals were sacrificed 24 h following pilocarpine administration as that is a period after which extensive SE-induced neuropathology is observed by hematoxylin and eosin (H&E) staining techniques in Li-pilo (Clifford et al., 1987; Fujikawa et al., 1999) and soman-induced convulsions in rats (McDonough et al., 1998). All animals were sacrificed by intraaortic perfusion-fixation while anesthetized with equithesin. The animals were initially perfused with heparinized phosphate buffered saline (PBS) (12.5 IU/ml, Sigma) followed by 10% formalin PBS (VWR Scientific Products). Brains were removed 4–6 days following perfusion and immersed in 10% formalin for a minimum of 24 h of postfixation. Following postfixation the brains were paraffin embedded and sectioned into 5 μm sections by a rotary microtome (Microm International). Brain sections were mounted on glass slides and stained with H&E.

Tissue sections were taken every 125 μm through the brain tissue 0.8–4.8 mm posterior to bregma (Paxinos and Watson, 1986). This specific brain region was chosen for analysis because it contains a preponderance of brain nuclei that exhibit the greatest degree of damage from soman (McDonough et al., 1998) and Li-pilo convulsions (Clifford et al., 1987; Fujikawa et al., 1999; Motte et al., 1998; Peredery et al., 2000). A scale of lesion severity developed for assessing soman toxicity (McDonough et al., 1989) was used to score the neuronal damage. The scale was as follows: 0 = none; 1 = minimal = ≤5% necrotic or malacic tissue; 2 = mild = 6–15% necrotic or malacic tissue; 3 = moderate = 16–40% necrotic or malacic tissue; 4 = severe = >40% necrotic or malacic tissue. All sections were rated by a single observer (JG) in a

blinded fashion. The mean damage score from a given region across all tissue sections was used as the neuronal damage score for statistical tests (McDonough et al., 1989).

Data and Statistical Analysis

Comparisons of the histopathological rating score parameters were performed using Kruskal–Wallis *H*-test for nonparametric statistical analysis. Statistical comparison of latency to SE onset, SE duration and spontaneous activity between groups was determined by analysis of variance (ANOVA) followed by Newman–Kuels post hoc test when a significant difference was determined by ANOVA. Values of $P < 0.05$ were considered significant for all statistical tests.

RESULTS

Li-pilo SE

A total of 65 rats completed the experimental paradigm and are included in the data analysis. Following pilocarpine administration, initial limbic seizure activity was followed by the onset of SE that is characterized by continuous high amplitude ECoG spiking (Ormandy et al., 1989) as shown in Fig. 1. The continuous high amplitude spiking typically continued for more than 2 h (Fig. 1).

ACPC and DCS Effect on Seizure Activity

Neither ACPC nor DCS induced any significant anticonvulsant effect on Li-pilo-induced SE. The average latency to SE onset in vehicle control animals was 33.8 min. The duration of SE as determined by continuous high frequency ECoG spiking in vehicle control animals was 138.4 min. Neither drug had any significant effect (one-way ANOVA) on the latency to SE when administered as exposure treatment immediately following the pilocarpine injection (Table 1). Likewise neither drug had any significant effect (one-way ANOVA) on SE duration whether administered as exposure treatment or following 5 min of SE (Table 1).

ACPC and DCS Effect on Neurological Deficit

Neither ACPC nor DCS had any significant effect on Li-pilo SE-induced neurological deficit as determined by spontaneous activity. The average distance traveled in the postseizure trial was in the range of 22–31% of the preseizure distance in vehicle control rats. The average resting time in the postseizure trial ranged from 199 to 220% of the preseizure time in vehicle control rats. The decreased activity 24 h following

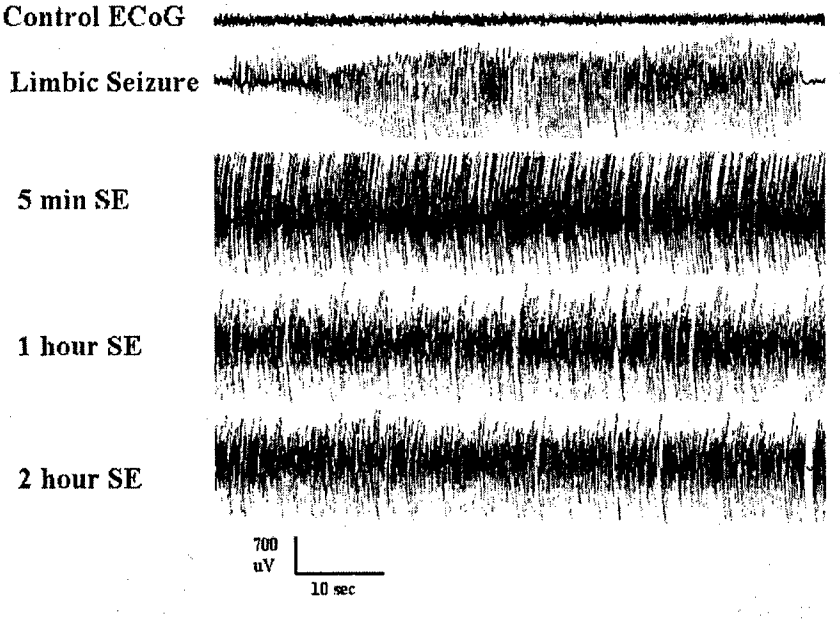

Fig. 1. Sample ECoG recording from Li-pilo-induced seizures. Initial limbic seizures characterized by chewing, nodding, forelimb clonus with rearing and falling were followed by continuous high amplitude ECoG spiking. During the SE the rats were immobile in an upright position with staring and slight tremor activity. The SE activity as defined electrographically typically continued for 2 h. All of the tracings shown here are from a single rat treated with 200 mg/kg ACPC i.p. 5 min following SE onset.

Table 1
Effect of ACPC and DCS on seizure activity

	Exposure treatment				
	Control ($n = 8$)	100 ACPC ($n = 7$)	200 ACPC ($n = 8$)	125 DCS ($n = 9$)	250 DCS ($n = 7$)
Latency to SE (min)	33.8 ± 1.5	37.6 ± 2.9	32.5 ± 1.58	38.0 ± 2.8	46.7 ± 5.9
Duration of SE (min)	138.6 ± 4.6	137.1 ± 11.0	148.3 ± 4.4	128.2 ± 4.4	127.0 ± 8.0
	5 min SE				
	Control ($n = 9$)	100 ACPC ($n = 7$)	200 ACPC ($n = 8$)	125 DCS ($n = 9$)	250 DCS ($n = 9$)
Latency to SE (min)	n/a	n/a	n/a	n/a	n/a
Duration of SE (min)	125.4 ± 2.7	135.6 ± 8.9	145.0 ± 6.8	133.3 ± 6.3	134.0 ± 3.7

pilocarpine administration was not significantly affected (one-way ANOVA) by either ACPC or DCS whether administered as exposure treatment or 5 min after SE onset (Table 2).

ACPC and DCS Effect on Neuropathology

As indicated in Table 3, the brain structures in the vehicle control groups demonstrated mean neural damage scores of 1.34–4.0 corresponding from 6% to greater than 40% necrotic or malacic tissue. These data indicate that the Li-pilo model of soman-induced SE in our hands induces a quantifiable degree of neuronal damage in the expected brain structures (Clifford et al., 1987; Fujikawa et al., 1999; McDonough et al., 1989, 1998). These data demonstrate that the 55 mg/kg dose of propofol administered to limit SE and improve 24 h survival also allowed the development of neuropathology comparable to that reported by others (Fujikawa et al., 1999; McDonough et al., 1998).

Significant neuroprotection was observed when ACPC was administered 5 min after the onset of Li-pilo-induced SE. The neuroprotection was most obvious in the temporal lobe regions as the macroscopic lesions induced by Li-pilo-induced SE were clearly reduced by ACPC administration (Fig. 2). As indicated in Table 3, both ACPC doses administered 5 min after the onset of SE significantly (Kruskal–Wallis H-test) reduced the neuropathology in all areas of the cortex (Fig. 3) as well as the cortical and lateral amygdala nuclei, amygdalopiriform and CA1 regions (Fig. 4). The differences between the two ACPC dose groups did not differ significantly. The 200 mg/kg ACPC dose also reduced the neuronal damage in the medial amygdala. Histologically brain regions from the vehicle control animals demonstrated dark basophilic shrunken nuclei with increased darkened cytoplasmic eosinophilia and vacuolation (Fig. 5). In areas affected by ACPC treatment 5 min after SE onset, the few affected cells contained darkened, occasionally angulated nuclei and slightly increased cytoplasmic eosinophilia and vacuolation (Fig. 5). Activity in the hippocampus was mixed with neuroprotection by 200 mg/kg ACPC in the CA1 (Fig. 4) but a significant increase by neuropathology ratings in the CA3 by the 100 mg/kg dose (Table 3).

ACPC exposure treatment induced little neuroprotective activity but appeared to exacerbate the Li-pilo-induced neuropathology in regions of the thalamus and hippocampus. ACPC exposure treatment (administered

Table 2
Effect of ACPC and DCS on spontaneous behavior

	Exposure treatment				
	Control ($n = 8$)	100 ACPC ($n = 7$)	200 ACPC ($n = 8$)	125 DCS ($n = 9$)	250 DCS ($n = 7$)
Distance traveled	22.4 ± 7.1	54.7 ± 20.2	68.1 ± 17.7	37.0 ± 15.8	26.6 ± 11.6
Resting time	219.5 ± 22.3	185.0 ± 37.0	169.3 ± 35.5	212.7 ± 31.1	256.3 ± 42.6
	5 min SE				
	Control ($n = 9$)	100 ACPC ($n = 7$)	200 ACPC ($n = 8$)	125 DCS ($n = 9$)	250 DCS ($n = 9$)
Distance traveled	31.4 ± 8.8	47.0 ± 15.6	78.9 ± 23.4	52.0 ± 20.0%	53.0 ± 10.5
Resting time	199.3 ± 28.3	199.9 ± 47.6	146.6 ± 33.6	192.0 ± 40.1	167.3 ± 37.5

Values are a percent of preSE control activity.

Table 3
Effects of ACPC and DCS on neural damage

Brain region	Exposure treatment				
	Control ($n = 8$)	100 ACPC ($n = 7$)	200 ACPC ($n = 8$)	125 DCS ($n = 9$)	250 DCS ($n = 7$)
Parietal cortex (Par1)	2.65	2.43	1.93	2.92	2.95
Occipital cortex (Oc)	2.69	2.21	1.74	2.54	2.59
Perirhinal cortex (PRh)	3.69	2.79	2.53[a]	3.74	3.61
Piriform cortex (Pir)	4.00	3.38	3.51	4.00	4.00
Cortical amygdala (Co)	3.91	3.71	3.57	3.83	3.68
Medial amygdala (BM)	3.16	3.14	3.01	3.26	3.31
Lateral amygdala (La)	3.58	3.46	3.51	3.89	3.85
Mediodorsal thalamus (MD)	2.25	3.49[a,b]	2.96[a,b]	2.93[a,b]	2.60
Lateraldorsal thalamus (LD)	2.25	3.33[a,b]	2.97[a,b]	2.87	2.58
Pretectal nucleus (APTD)	2.13	2.90[a,b]	2.62[a,b]	2.99[a,b]	2.39
CA3	2.59	3.35[a,b]	3.02[a,b]	2.66	2.41
CA1	1.34	1.22	1.25	1.27	1.03
Amygdalopiriform (Apir)	4.00	3.98	3.82	4.00	4.00
	5 min SE				
	Control ($n = 9$)	100 ACPC ($n = 7$)	200 ACPC ($n = 8$)	125 DCS ($n = 9$)	250 DCS ($n = 9$)
Parietal cortex (Par1)	3.00	1.50[a]	1.29[a]	2.54	2.54
Occipital cortex (Oc)	2.80	1.32[a]	1.30[a]	2.44	2.40
Perirhinal cortex (PRh)	3.59	1.89[a]	1.86[a]	3.16	3.42
Piriform cortex (Pir)	3.97	3.16[a]	2.85[a]	3.83	3.98
Cortical amygdala (Co)	4.00	3.30[a]	3.44[a]	3.97	3.94
Medial amygdala (BM)	3.44	3.03	2.54[a]	3.44	3.43
Lateral amygdala (La)	3.76	3.36[a]	3.04[a]	3.73	3.81
Mediodorsal thalamus (MD)	3.09	2.87	2.52	2.94	2.89
Lateraldorsal thalamus (LD)	3.20	2.98	2.53	3.05	3.12
Pretectal nucleus (APTD)	2.74	2.74	2.10	2.79	2.66
CA3	2.81	3.32[a,b]	2.89	2.79	2.72
CA1	1.46	1.09	0.94[a]	1.24	1.28
Amygdalopiriform (Apir)	4.00	3.26[a]	3.63[a]	3.97	4.00

[a] Indicates statistically significant difference from control group as determined by Kruskal–Wallis H-test.
[b] Indicates that neuronal damage was enhanced by ACPC or DCS treatment.

immediately following pilocarpine) induced neuroprotection only in the perirhinal cortex at the 200 mg/kg dose (Table 3). In contrast, both doses of ACPC administered as exposure treatment significantly enhanced neuronal damage (Kruskal–Wallis H-test) in the mediodorsal thalamus, laterodorsal thalamus, pretectal nucleus and the hippocampal CA3 region (Table 3; Fig. 6). The enhanced neurodamage was observed as an increase in the number of necrotic neurons with dark basophilic shrunken and sometimes angulated nuclei with increased cytoplasmic eosinophilia and vacuolation (Fig. 7).

Treatment with DCS either at exposure or following 5 min SE had no significant effect on the Li-pilo-induced neuropathology in most brain regions (Table 3). The exceptions were the mediodorsal thalamus and pretectal nucleus in the 125 mg/kg DCS exposure treatment group in which the neuropathology was significantly greater (Kruskal–Wallis H-test) than the vehicle control group (Table 3). The appearance of the lesions in the mediodorsal thalamus and pretectal nucleus associated with DCS were histologically similar to those induced by ACPC as illustrated in Fig. 7.

DISCUSSION

ACPC produced significant neuroprotection in Li-pilo convulsions when administered 5 min following SE onset. ACPC reduced by half the mean neural damage scores induced by 3 h SE in the parietal, occipital and perirhinal cortices. Significant reductions were also produced in the piriform cortex, amygdala and CA1 region of the hippocampus. This collaborates previous reports of ACPC neuroprotection in other experimental models including primary neuronal cell cultures (Boje et al., 1993; Fossom et al., 1995), cerebral ischemia (Fossom et al., 1995) and dynorphin A-induced spinal

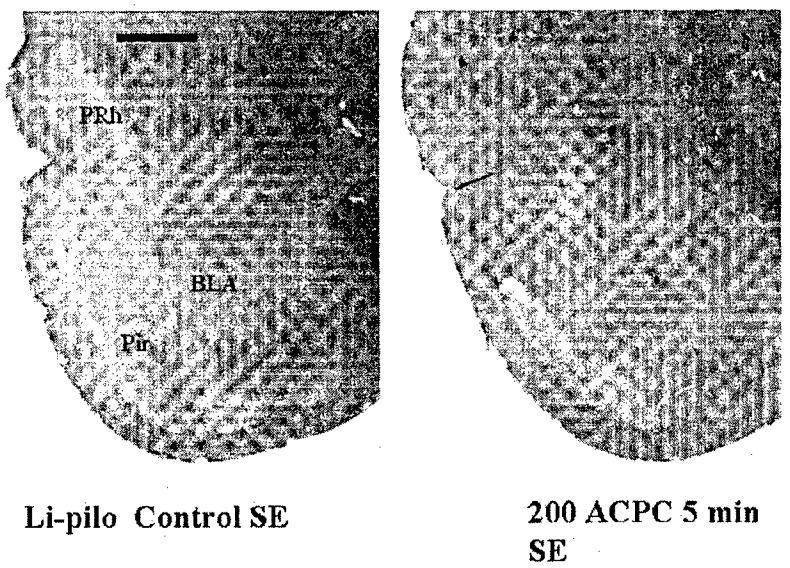

Fig. 2. H&E stained sections of the rat temporal lobe showing the macroscopic lesions resulting from Li-pilo-induced convulsions and the neuroprotection induced by ACPC administered 5 min after the onset of SE. PRh: perirhinal cortex, Pir: piriform cortex, BLA: basolateral amygdala. Scale bar represents 1 mm.

injury (Long and Skolnick, 1994). The neuroprotection resulted from a single ACPC administration whereas the neuroprotection in Li-pilo SE by vigabatrin (Andre et al., 2001) or caffeine (Rigoulot et al., 2003) required chronic administration. Given that the duration of pharmacological activity following i.p. administration in rats is approximately 1 h (Smith et al., 1993), we predict that repeated ACPC administrations would produce even greater neuroprotection during the 3 h duration SE that was tested in this experiment. The brief duration of action would also explain the lack of neuroprotection when ACPC was administered as exposure treatment. Finally, ACPC was neuroprotective without affecting ongoing seizure activity. Pharmacologically induced neuroprotection without anticonvulsant activity has been reported in Li-pilo SE (Andre et al., 2001; Rigoulot et al., 2003), kainic acid SE (Rong et al., 1999) and soman SE (Filbert et al., 1999). This suggests that the mechanisms of SE-induced seizure activity and neuronal damage are linked but independent.

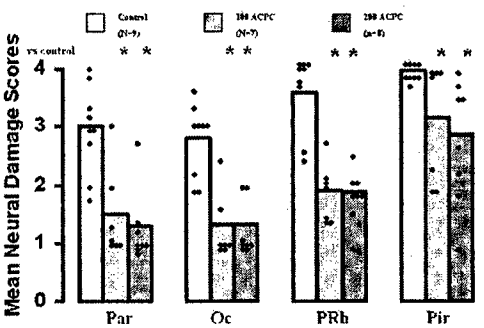

Fig. 3. Graphic presentation of the neuroprotection induced by ACPC when administered 5 min after the onset of SE. Both the individual neuropathology scores and the group average are depicted. Considerable reduction of neuropathology was observed in the parietal, occipital and perirhinal cortices regardless of the dose. Note that neuronal damage was enhanced in CA3 by the 100 mg/kg ACPC dose. Asterisk (*) indicates significant difference from the corresponding Li-pilo control group as determined by Kruskal–Wallis H-test. Abbreviations are as indicated in Table 1.

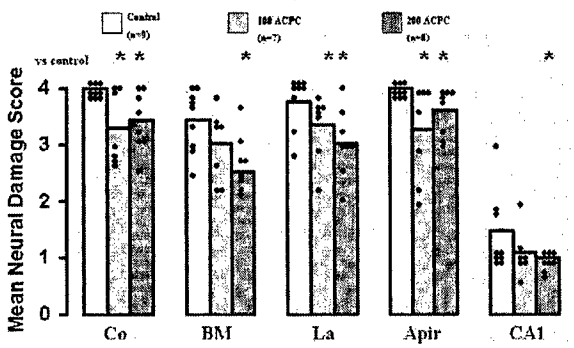

Fig. 4. Graphic presentation of the effects of ACPC on neuronal damage when administered 5 min after the onset of SE. Although more resistant to the single dose ACPC effects than cortical areas, neuroprotection was also observed in the amygdala and CA1 regions. Asterisk (*) indicates significant difference from the corresponding Li-pilo control group as determined by Kruskal–Wallis H-test. Abbreviations are as indicated in Table 1.

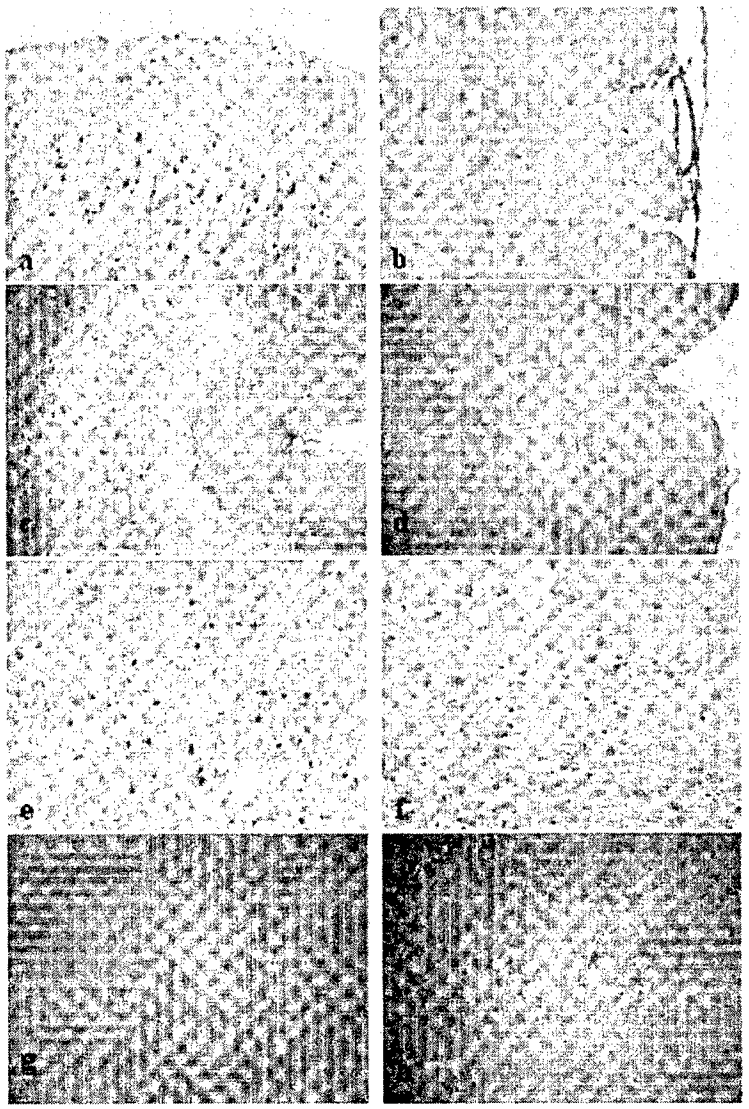

Fig. 5. Representative high magnification H&E sections of rat brains showing the severity of Li-pilo-induced neuropathology and examples of the neuroprotection induced by ACPC. (a) Parietal cortex from control showing moderate necrosis of cortical neurons (16–40% cells necrotic). The affected neurons contain dark basophilic shrunken angulated nuclei with increased darkened cytoplasmic eosinophilia and vacuolation (200×). (b) Parietal cortex from ACPC treatment begun 5 min after SE showing minimal necrosis of cortical neurons (1–5% cells necrotic). The few affected cells contain dark shrunken nuclei with increased darkened cytoplasmic eosinophilia and vacuolation (200×). (c) Perirhinal cortex from control showing severe necrosis of cortical region (>40% cells necrotic). The affected cells are pyknotic and karyorrhectic with loss of cytoplasmic detail. Vacuolation, intercellular edema and malacia are extensive paralleling the adjacent sulcus (100×). (d) Perirhinal cortex from ACPC treatment begun 5 min after SE showing minimal necrosis of neurons (1–5% cells necrotic). The few affected cells contain darkened occasionally angulated nuclei, slightly increased cytoplasmic eosinophilia and vacuolation (100×). (e) Lateral amygdala from control showing severe necrosis (>40% cells necrotic). The affected cells contain dark shrunken nuclei and increased cytoplasmic eosinophilia or loss of cytoplasmic detail. Vacuolation, intercellular edema, malacia and occasional segmented leukocytes were also visible (200×). (f) Lateral amygdala from ACPC treatment begun 5 min after SE showing moderate necrosis (16–40% cells necrotic). The necrotic cells contain shrunken basophilic nuclei and the cytoplasms contain increased eosinophilia and vacuolation. The clear areas between cells indicate intercellular edema (200×). (g) Amygdalopiriform from control showing severe necrosis (>40% cells necrotic). The affected cells contain dark pyknotic nuclei with increased eosinophilia and vacuolated cytoplasms. There is also intercellular edema and early malacia evident (100×). (h) Amygdalopiriform from ACPC treatment began 5 min after SE showing moderate necrosis (16–40% cells necrotic). The necrotic cells contain dark shrunken nuclei and increased eosinophilia and vacuolation of cytoplasm (100×).

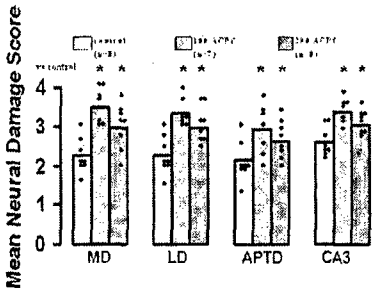

Fig. 6. Graphic presentation of the exacerbated neuronal damage induced by ACPC when administered as exposure treatment. Although only ACPC is depicted here, both ACPC and DCS enhanced neuropathology observed in the thalamus. ACPC also enhanced neuronal damage in CA3. Asterisk (*) indicates significant difference from the corresponding vehicle control group as determined by Kruskal–Wallis *H*-test. Abbreviations are as indicated in Table 1.

In contrast to ACPC, DCS had no neuroprotective activity in Li-pilo-induced SE. Both DCS (Henderson et al., 1990; Hood et al., 1989) and ACPC (Marvizon et al., 1989; Watson and Lanthorn, 1990) were originally identified as high efficacy partial agonists of the strychnine-insensitive glycine site. However, clear differences between the two drugs in anticonvulsant activity (Bisaga et al., 1993), anxiolytic activity (Karcz-Kubicha et al., 1997) and neuroprotection in cell culture (Boje et al., 1993) have been reported in addition to the differential in vivo neuroprotection observed in this study. Additional differences in the mechanisms of pharmacological activity have been identified using NMDA receptors expressed in *Xenopus* oocytes where ACPC has been shown to be both a full agonist of the strychnine-insensitive glycine site and a low affinity competative NMDA antagonist (Nahum-Levy et al., 1999). The degree of interaction between the opposing ACPC effects depends on the subunit composition of the NMDA receptors in a particular region (Sheinin et al., 2002). In contrast, DCS acts only at the glycine site but may have either greater or less efficacy than glycine depending on the NMDA receptor subunit composition (Sheinin et al., 2001). These findings indicate that glutamate antagonism is critical for the ACPC-induced neuroprotection while specific activity at the glycine site, as is the case for DCS, provides no neuroprotection in Li-pilo SE.

ACPC produced significant neuroprotection in the cortical and amygdala regions. The competitive NMDA antagonist CGP 40116 (Fujikawa et al., 1994) and noncompetitive NMDA antagonist ketamine (Fujikawa, 1995) also produced significant neuroprotection in cortical areas in Li-pilo SE. In contrast, caffeine (Rigoulot et al., 2003) and vigabatrin (Andre et al., 2001) either had no neuroprotective effect or exacerbated Li-pilo neuronal damage in cortical areas. Taken together the evidence would suggest that NMDA antagonism is necessary for cortical neuroprotection. The predominance of NMDAR2A receptors in cortical areas (Ishii et al., 1993) where ACPC has greater competitive NMDA antagonist activity (Sheinin et al., 2002) may explain the ACPC neuroprotection observed there.

ACPC and DCS administered as exposure treatment exacerbated the neuropathology observed in thalamic regions. Although NMDA antagonists induce limbic neuropathology (Olney et al., 1991) this is not the case with ACPC and DCS (Berger et al., 1994). We hypothesize that the variable effect of ACPC and DCS on neuropathology is due to regional differences in NMDA receptor subtype distribution (Ishii et al., 1993). For example, the enhanced thalamic neuropathology may result from the presence of NMDAR2B and 2C receptors (Ishii et al., 1993) where ACPC exerts less competitive NMDA antagonist and more glycine agonist activity that would enhance NMDA receptor excitotoxicity (Nahum-Levy et al., 1999; Sheinin et al., 2002). The thalamus also contains a significant proportion of NMDAR2C receptors (Ishii et al., 1993) where DCS exerts greater than full agonist activity at the strychnine-insensitive glycine sites thereby facilitating excitotoxicity in that region (Newell et al., 1997; Sheinin et al., 2001). Interestingly, the enhanced neuropathology following ACPC exposure treatment, but not following SE onset administration, suggests different populations of NMDA receptors may be involved at different times during SE.

ACPC administered after 5 min of SE or as exposure treatment potentiated the neuropathology in CA3 (Figs. 2 and 4) but was neuroprotective in CA1 (Fig. 2). Nonuniform CA3 and CA1 regional responses to neuroprotective drug treatments have also been reported following caffeine (Rigoulot et al., 2003) and vigabatrin (Andre et al., 2001) treatment in Li-pilo seizures. This evidence supports a hypothesis of regional differences in the mechanism of neuropathology in Li-pilo-induced SE (Rigoulot et al., 2003). In the case of ACPC the regional responses may be related to the distribution of hippocampal NMDAR2A and 2B receptors (Ishii et al., 1993) where ACPC has variable degrees of activity as both a glycine site agonist and competative NMDA antagonist (Newell et al., 1997; Sheinin et al., 2002).

The reduced spontaneous motor activity observed 24 h following pilocarpine administration was not

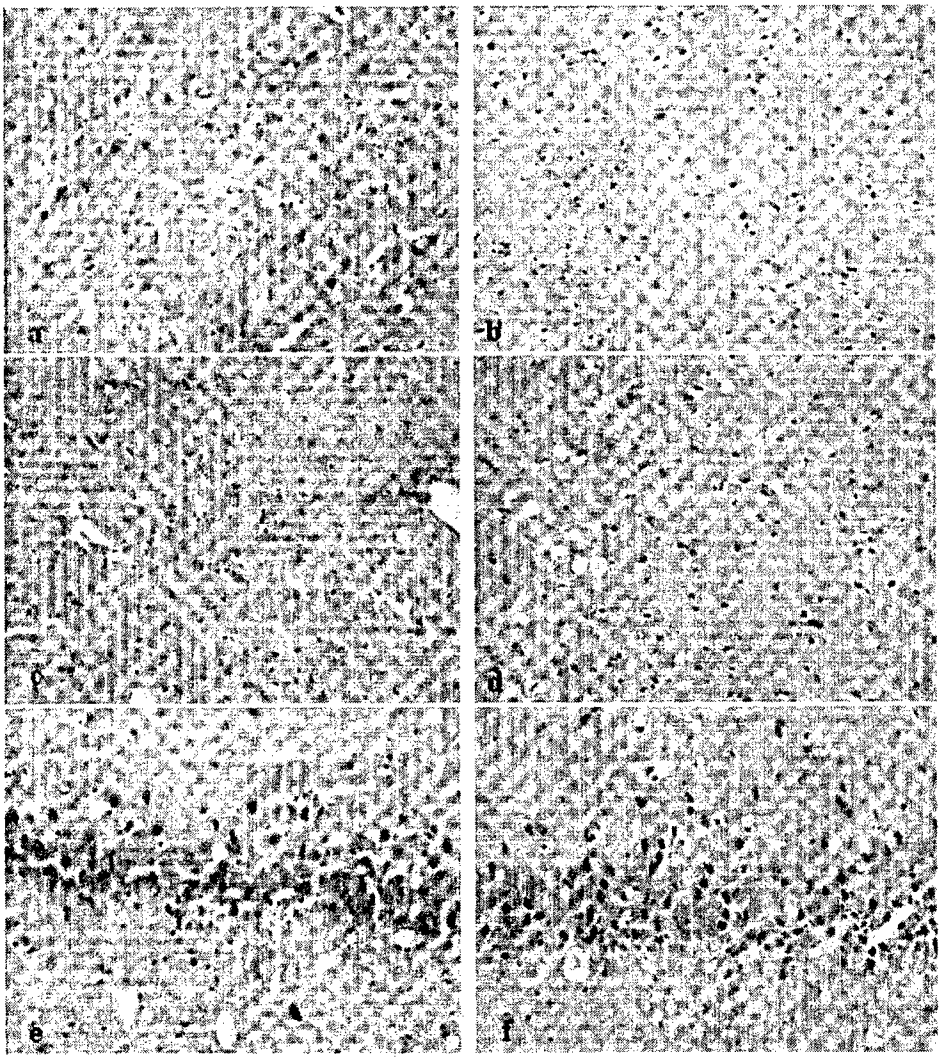

Fig. 7. Representative H&E sections of rat brains demonstrating the enhancement of Li-pilo-induced neuropathology by ACPC and DCS. (a) Mediodorsal thalamus from control showing mild necrosis (6–15% cells necrotic). The affected neurons contain basophilic slightly shrunken angulated nuclei with increased cytoplasmic eosinophilia and vacuolation (200×). (b) Mediodorsal thalamus from ACPC exposure treatment showing severe necrosis (>40% cells necrotic). The affected neurons contain dark basophilic shrunken sometimes angulated nuclei with increased cytoplasmic eosinophilia, vacuolation and loss of cytoplasmic detail (200×). (c) Pretectal nucleus from control showing mild necrosis (6–15% cells necrotic). The necrotic cells contain shrunken basophilic nuclei with increased cytoplasmic eosinophilia and vacuolation (200×). (d) Pretectal nucleus from ACPC treatment showing severe necrosis (>40% cells necrotic). The necrotic cells contain shrunken basophilic angulated nuclei with increased cytoplasmic eosinophilia and vacuolation (200×). (e) CA3 from control showing moderate necrosis (16–40% cells necrotic). The necrotic cells contain shrunken basophilic nuclei and increased cytoplasmic eosinophilia and vacuolation (200×). (f) CA3 from ACPC exposure treatment showing severe necrosis (>40% cells necrotic). The necrotic cells contain basophilic angulated nuclei with increased cytoplasmic eosinophilia and vacuolation (200×).

affected in rats demonstrating significant ACPC neuroprotection. This supports a previous report that morphological neuroprotection is not well correlated with recovery of behavioral activities when tested immediately following pilocarpine-induced SE (Hort et al., 1999). The onset of spontaneous recurrent seizures also is used as a measure of behavioral or functional neuroprotection. Significant levels of morphological neuroprotection induced by pharmacological agents or kindled amygdala seizures did not affect the onset of spontaneous recurrent seizures following Li-pilo-induced SE (Andre et al., 2001; Rigoulot et al., 2003). Further studies are required to determine effects of ACPC-induced neuroprotection on long-term

cognitive function and the development of spontaneous recurrent seizures following Li-pilo SE.

DCS inhibits kainic acid-induced convulsions (Baran et al., 1994). In the present study DCS doses in the same range as those effective in kainic acid convulsions (Baran et al., 1994) had no anticonvulsant or neuroprotective effect in Li-pilo-induced SE. This discrepancy may be a result of the present study evaluating multiple parameters of SE activity including electrographic ECoG seizure activity, latency to SE, SE duration and neuropathology while the kainate study evaluated only behavioral convulsions (Baran et al., 1994). Alternatively, the differential DCS effect may result from fundamental differences in the mechanism of kainic acid and Li-pilo-induced seizures. Kainic acid is hypothesized to act presynaptically to induce glutamate release (Chittajally et al., 1996; Ferkany et al., 1982; Liu et al., 1997) while pilocarpine and other cholinergic convulsants activate muscarinic receptors that induce glutamate release (Jobe et al., 1986; McDonough and Shih, 1997; Ormandy et al., 1989; Solberg and Belkin, 1997). Because glutamate antagonists inhibit both kainic acid (Clifford et al., 1990) and Li-pilo convulsions (Ormandy et al., 1989; Walton and Treiman, 1991) we propose that different subsets of NMDA receptors are involved in the two seizure types and that DCS is active only with NMDA receptors involved in kainic acid SE (Ishii et al., 1993; Sheinin et al., 2001).

In conclusion, ACPC induced significant neuroprotection when administered at the onset of Li-pilo-induced SE. Due to a short duration of action, we propose that repeated ACPC doses would enhance the neuroprotection. The observed effects were nonuniform, with neuroprotection in the amygdala and cortical regions and an exacerbation of neuronal damage in the thalamus. The differential effect on neural damage is proposed to result from regional differences in NMDA receptor subunit composition that are variably affected by ACPC. The heterogeneity of NMDA receptor expression may also explain the lack of activity by DCS. Further studies are required to determine the effects of ACPC neuroprotection on the sequela of Li-pilo SE and how those effects may relate to the treatment of organophosphorous nerve agent exposure.

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REFERENCES

Andre A, Ferrandon A, Marescaux C, Nehlig A. Vigabatrin protects against hippocampal damage but is not antiepileptogenic in the lithium-pilocarpine model of temporal lobe epilepsy. Epilepsy Res 2001;47:99–117.

Baran H, Loscher W, Mevissen M. The glycine/NMDA receptor partial agonist D-cycloserine blocks kainate-induced seizures in rats. Comparison with MK-801 and diazepam. Brain Res 1994; 652:195–200.

Berger P, Farrel K, Sharp F, Skolnick P. Drugs acting at the strychnine-insensitive glycine receptor do not induce HSP-70 protein in the cingulated cortex. Neurosci Lett 1994;168:147–50.

Bisaga A, Krascik P, Jankowska E, Palejko W, Kostowski W, Danysz W. Effect of glutamate receptor antagonists on N-methyl-D-aspartate and (*S*)-α-amino-3-hydoxy-5-methyl-4-isoxazolepropionic acid induced convulsant effects in mice and rats. Eur J Pharmacol 1993;242:213–20.

Boje KM, Wong G, Skolnick P. Desensitization of the NMDA receptor complex by glycinergic ligands in cerebellar granule cell cultures. Brain Rés 1993;603:207–14.

Bonhaus D, Burge B, McNamara J. Biochemical evidence that glycine allosterically regulates an NMDA receptor-coupled ion channel. Eur J Pharmacol 1987;142:489–90.

Bowery N. Glycine binding sites and NMDA receptors in the brain. Nature 1987;326:338.

Chittajally R, Vignes M, Dev KK, Barnes JM, Collingridge GL, Henley JM. Regulation of glutamate release by presynaptic kainite receptors in the hippocampus. Nature 1996;379:78–81.

Clifford DB, Olney JW, Maniotis A, Collins RC, Zorumski CF. The functional anatomy and pathology of lithium-pilocarpine and high-dose pilocarpine seizures. Neuroscience 1987;23(3):953–68.

Clifford DB, Olney JW, Benz AM, Fuller TA, Zorumski CF. Ketamine, phencyclidine, and MK-801 protect against kainic acid-induced seizure-related brain damage. Epilepsia 1990; 31(4):382–9.

Dubé C, Boyet S, Marescaux C, Nehlig A. Progressive metabolic changes underlying the chronic reorganization of brain circuits during the silent phase of the lithium-pilocarpine model of epilepsy in the immature and adult rat. Exp Neurol 2000;162: 146–57.

Dubé C, Boyet S, Marescaux C, Nehlig A. Relationship between neuronal loss and interictal glucose metabolism during the chronic phase of the lithium-pilocarpine model of epilepsy in the immature and adult rat. Exp Neurol 2001;167:227–41.

Ferkany JW, Zaczek R, Coyle JT. Kainic acid stimulates excitatory amino acid neurotransmitter release at presynaptic receptors. Nature 1982;298:757–9.

Filbert MG, Forster JS, Smith CD, Ballough GPH. Neuroprotective effects of HU-211 on brain damage resulting from soman-induced seizures. Ann NY Acad Sci 1999;890:505–14.

Fossom LH, Von Lubitz DKJE, Lin RCS, Skolnick P. Neuroprotective actions of 1-aminocyclopropanecarboxylic acid (ACPC): a partial agonist at strychnine-insensitive glycine sites. Neurol Res 1995;17:265–9.

Fujikawa DG. Neuroprotecitve effect of ketamine administered after status epilepticus onset. Epilepsia 1995;36(2):186–95.

Fujikawa DG, Daniels AH, Kim JS. The competitive NMDA receptor antagonist CGP 40116 protects against status epilepticus-induced neuronal damage. Epilepsy Res 1994;17: 207–19.

Fujikawa DG, Shinmei SS, Cai B. Lithium-pilocarpine-induced status epilepticus produces necrotic neurons with internecleosomal DNA fragmentation in adult rats. Eur J Neurosci 1999; 11:1605–14.

Henderson G, Johnson JW, Ascher P. Competitive antagonists and partial agonists at the glycine modulatory site of the mouse *N*-methyl-D-aspartate receptor. J Physiol 1990;430:189–212.

Honchar MP, Olney JW, Sherman WR. Systemic cholinergic agents induce seizures and brain damage in lithium-treated rats. Science 1983;220:323–34.

Hood WF, Compton RP, Monahan JB. D-cycloserine: a ligand for the *N*-methyl-D-aspartate coupled glycine receptor has partial agonist characteristics. Neurosci Lett 1989;98:91–5.

Hort J, Brozek G, Mares P, Langmeier M, Komarek V. Cognitive functions after pilocarpine-induced status epilepticus: changes during silent period precede appearance of spontaneous recurrent seizures. Epilepsia 1999;40(9):1177–83.

Ishii T, Moriyoshi K, Sugihara H, Sakurada K, Kadotani H, Yokoi M et al. Molecular characterization of the family of the *N*-methyl-D-aspartate receptor subunits. J Biol Chem 1993; 268(4):2836–43.

Jobe RS, Morrisett RA, Snead OC. Characterization of lithium potentiation of pilocarpine-induced status epilepticus in rats. Exp Neurol 1986;91:471–80.

Johnson J, Ascher P. Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 1987;325:529–31.

Karcz-Kubicha M, Jessa M, Nazar M, Plaznik A, Hartmann S, Parsons CG et al. Anxiolytic activity of glycine-B antagonists and partial agonists. No relation to intrinsic activity in the patch clamp. Neuropharmacology 1997;36(10):1355–67.

Kemp JA, Foster AC, Leeson PD, Priestley T, Tridgett R, Iversen LL et al. 7-Chlorokynurenic acid is a selective antagonist at the glycine modulatory site of the *N*-methyl-D-aspartate receptor complex. Proc Natl Acad Sci USA 1988;85:6547–50.

Kleckner NW. Dingledine R. Requirement for glycine in activation of NMDA-receptors expressed in *Xenopus* oocytes. Science 1988;241:835–7.

Kleckner NW, Dingledine R. Selectivity of quinoxalines and kynurenines as antagonists of the glycine site on *N*-methyl-D-aspartate receptors. Mol Pharmacol 1989;36:430–6.

Liu Z, Stafstrom CE, Sarkisian MR, Yang Y, Hori A, Tandon P et al. Seizure-induced glutamate release in mature and immature animals; an in vivo microdialysis study. NeuroReport 1997;8(8):2019–23.

Long JB, Skolnick P. 1-Aminocyclopropanecarboxylic acid protects against dynorphin A-induced spinal injury. Eur J Pharmacol 1994;261:295–301.

Loscher W, Wlaz P, Rundfeldt C, Baran H, Honack D. Anticonvulsant effects of the glycine/NMDA receptor ligands D-cycloserine and D-serine but not R-(+)-HA-966 in amygdala-kindled rats. Br J Pharmacol 1994;112:97–106.

Marvizon JCG, Lewin AH, Skolnick P. 1-Aminocyclopropanecarboxylic acid: a potent and selective ligand for the glycine modulatory site of the *N*-methyl-D-aspartate receptor complex. J Neurochem 1989;52:992–4.

McDonough JH, Shih TM. Pharmacological modulation of soman-induced seizures. Neurosci Biohehav Rev 1993;17:203–15.

McDonough JH, Shih TM. Neuropharmacological mechanisms of nerve agent-induced seizure and neuropathology. Neurosci Biobehav Rev 1997;21(5):559–79.

McDonough JH, Jaax NK, Crowley RA, Mays MZ, Modrow HE. Atropine and/or diazepam therapy protects against soman-induced neural and cardiac pathology. Fundam Appl Toxicol 1989;13:256–76.

McDonough JH, Clark TR, Slone TW, Zoeffel D, Brown K, Kim S et al. Neural lesions in the rat and their relationship to EEG delta activity following seizures induced by the nerve agent soman. NeuroToxicology 1998;19(3):381–92.

McLean MJ, Gupta RC, Dettbarn WD, Wamil AW. Prophylactic and therapeutic efficacy of memantine against seizures produced by soman in the rat. Toxicol Appl Pharmacol 1992;112:95–103.

Morrisett RA, Jope RS, Snead OC. Effects of drugs on the initiation and maintenance of status epilepticus induced by administration of pilocarpine to lithium-pretreated rats. Exp Neurol 1987;97:193–200.

Motte J, da Silva Fernandes MJ, Baram TZ, Nehlig A. Spatial and temporal evolution of neuronal activation, stress and injury in lithium-pilocarpine seizures in adult rats. Brain Res 1998;798: 61–72.

Nahum-Levy R, Fossom LH, Skolnick P, Benveniste M. Putative partial agonist 1-aminocyclopropanecarboxylic acid acts concurrently as a glycine-site agonist and a glutamate-site antagonists at *N*-methyl-D-aspartate receptors. Mol Pharmacol 1999;56:1207–18.

Newell DW, Barth A, Ricciardi TN, Malouf AT. Glycine causes increased excitability and neurotoxicity by activation of NMDA receptors in the hippocampus. Exp Neurol 1997;145:235–44.

Olney JW, Labruyere J, Wang G, Wozniak DF, Price MT, Sesma MA. NMDA antagonist neurotoxicity: mechanism and prevention. Science 1991;254:1515–8.

Ormandy GC, Jope RS, Snead OC. Anticonvulsant actions of MK-801 on the lithium-pilocarpine model of status epilepticus in rats. Exp Neurol 1989;106:172–80.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. Orlando, FL: Academic Press; 1986.

Peredery O, Persinger MA, Parker G, Mastrosov L. Temporal changes in neuronal dropout following inductions of lithium/pilocarpine seizures in the rat. Brain Res 2000;881:9–17.

Peterson SL. 7-chlorokynurenic acid antagonizes the anticonvulsant activity of D-cycloserine in maximal electroshock seizures. Epilepsy Res 1992;13:73–81.

Rigoulot MA, Leroy C, Koning E, Ferrandon A, Mehlig A. Prolonged low-dose caffeine exposure protects against hippocampal damage but not against the occurrence of epilepsy in the lithium-pilocarpine model in the rat. Epilepsia 2003; 44(4):529–35.

Rogawski MA. The NMDA receptor, NMDA antagonists, and epilepsy therapy. Drugs 1992;44(3):279–92.

Rong Y, Doctrow SR, Tocco G, Baudry M. EUK-134, a synthetic superoxide dismutase and catalase mimetic, prevents oxidative stress and attenuates kainate-induced neuropathology. Proc Natl Acad Sci USA 1999;96:9897–902.

Sheinin A, Shavit S, Benveniste M. Subunit specificity and mechanism of action of NMDA partial agonist D-cycloserine. Neuropharmacology 2001;41:151–8.

Sheinin A, Nahum-Levy R, Shavit S, Benveniste M. Specificity of putative partial agonist, 1-aminocyclopropanecarboxylic acid for rat *N*-methyl-D-aspartate receptor subunits. Neurosci Lett 2002;317:77–80.

Shih TM. Anticonvulsant effects of diazepam and MK-801 in soman poisoning. Epilepsy Res 1990;7:105–16.

Skolnick P, Marvizon JCG, Jackson BW, Monn JA, Rice KC, Lewin AH. Blockade of the *N*-methyl-D-aspartate induced convulsions by 1-aminocyclopropanecarboxylates. Life Sci 1989;45:1647–55.

Smith SC, Al-Zubaidy ZA, Chapman AG, Meldrum BS. Excitatory amino acid antagonists, lamotrigine and BW 1003C87 as anticonvulsants in the genetically epilepsy-prone rat. Epilepsy Res 1993;15:101–11.

Solberg Y, Belkin M. The role of excitotoxicity in organophosphorous nerve agents central poisoning. TIPS 1997;18(6):183–5.

Walton NY, Treiman DM. Motor and electroencephalographic response of refractory experimental status epilepticus in rats to treatment with MK-801, diazepam, or MK-801 plus diazepam. Brain Res 1991;553:97–104.

Watson GB, Lanthorn TH. Pharmacological characteristics of cyclic homologues of glycine at the *N*-methyl-D-aspartate receptor-associated glycine site. Neuropharmacology 1990;29(8):727–30.

White HS, Woodhead JH, Franklin MR, Swinyard EA, Wolf HH. Experimental selection, quantification, and evaluation of antiepileptic drugs. In: Levy HH, Mattson RH, Meldrum BS, editors. Antiepileptic drugs. New York: Raven Press; 1995. p. 99–110.

Wlaź P. Anti-convulsant and adverse effects of the glycine$_B$ receptor ligands, D-cycloserine and L-701, 324: comparison with competitive and non-competitive *N*-methyl-D-aspartate receptor antagonists. Brain Res Bull 1998;46(6):535–40.

Anticonvulsant and Neuroprotective Effects of Propofol in Cholinergic Status Epilepticus

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Propofol is a nonbarbiturate anesthetic with anticonvulsant activity. In addition to being a $GABA_A$ agonist, propofol has NMDA antagonist and antioxidant properties, would be a potential benefit in the prevention of status epilepticus (SE)-induced neuropathology. The anticonvulsant and neuroprotectant activity of propofol was tested in the lithium-pilocarpine (Li-pilo) model of organophosphorus nerve agent induced-SE. Male rats were surgically implanted with cortical electrodes that allowed continuous recording of electrocorticogram (ECoG) activity. The animals were sacrificed 24 hours following pilocarpine administration and the brains prepared for pathological analysis (hematoxylin and eosin stain). Propofol (55 mg/kg, i.p.) prevented all motor and electrographic SE activity and related neuropathology when administered immediately following pilocarpine exposure (n=5). Administered after 5 minutes (n=5) or 3 hours of Li-pilo-induced SE (n=5), propofol (55 mg/kg, i.p.) inhibited all motor and electrographic seizure activity in addition to reducing mortality such that all rats survived the 24 hour period following pilocarpine administration. Propofol (55 mg/kg, i.p.) administered following 5 minutes of Li-pilo SE completely suppressed ECoG spiking in an average of 12.2 minutes, significantly less than the 20.8 minutes required by the same dose following 3 hours SE (t-test, $P<0.05$). This supports previous evidence that late stage SE is more difficult to treat. No neuropathology was observed in the rats treated with propofol (55 mg/kg, i.p.) 5 minutes following SE onset. Rats (n=5) experiencing 3 hours of SE prior to propofol (55 mg/kg, i.p.) demonstrated significant neuropathology in the piriform and perirhinal cortices. However, rats (n=5) administered an additional 20 mg/kg (i.p.) 2 hours following the initial 55 mg/kg propofol dose exhibited significantly less neuropathology than those receiving only the initial dose as quantified by necrosis scores (Mann-Whitney U-test, $P<0.05$) and lesion volume analysis (ANOVA, $P<0.05$). The neuropathology in the propofol extended treatment animals (55 mg/kg plus 20 mg/kg) was not significantly different from seizure free rats. Overall, propofol induced significant anticonvulsant and neuroprotectant activity even following 3 hours of continuous SE. This contrasts with diazepam and midazolam which must be administered in the initial minutes of SE to induce anticonvulsant activity. The anticonvulsant and neuroprotectant benefit of the propofol extended treatment may result from NMDA antagonist and antioxidant activity. Because Li-pilo SE shares many characteristics of the treatment resistant cholinergic convulsions induced by

organophosphorus nerve agents, the results of this study suggest propofol may serve as an effective treatment for nerve agent exposure.

INTRODUCTION

Pretreatment of rats with lithium chloride 20-24 hours prior to pilocarpine administration produces status epilepticus (SE) of several hours duration (Honchar et al., 1983; Clifford et al., 1987). If allowed to proceed the SE produces well-characterized lesions in the cortex, thalamus, hippocampus and amygdala regions (Honchar et al., 1983; Clifford et al., 1987; Motte et al., 1998; Peredery et al., 2000; Dube et al., 2000; Dube et al., 2001). Following a latent period of several weeks the animals that experience SE-induced neuropathology develop spontaneous limbic seizures (Lemos and Cavalheiro, 1995; Dube et al., 2000; Dube et al., 2001; Glien et al., 2001).

Lithium-pilocarpine (Li-pilo) convulsions model the cholinergic convulsions induced by the organophosphorus nerve agent soman. Both Li-pilo (Jobe et al., 1986; Morrisett et al., 1987) and soman convulsions (McDonough and Shih, 1993; Shih et al., 1997; McDonough and Shih, 1997) have an initial cholinergic phase that is inhibited by muscarinic antagonists. Both Li-pilo (Ormandy et al., 1989; Walton and Treiman, 1991) and soman convulsions (Shih, 1990; McLean et al., 1992; McDonough and Shih, 1993; McDonough and Shih, 1997) have a secondary glutamatergic phase that is inhibited by glutamate antagonists (McDonough and Shih, 1997; Solberg and Belkin, 1997). Both produce a similar pattern of neuropathology that is particularly severe in the piriform and entorhinal cortices (Clifford et al., 1987; McDonough et al., 1998; Motte et al., 1998; Peredery et al., 2000). Finally, both Li-pilo (Jope et al., 1986; Morrisett et al., 1987) and soman convulsions (Shih et al., 1997; McDonough and Shih, 1997) are relatively

refractory to drug therapy with the additional confound that the longer the SE continues the more difficult pharmacological treatment becomes (Walton and Treiman, 1988; Jones et al., 2002).

Propofol is a highly lipid soluble, nonbarbiturate anesthetic that possess anticonvulsant activity. Low doses disrupt ictal-like discharge in rat hippocampal slices (Rasmussen et al., 1996). In rodents, propofol inhibits seizures induced by electroshock, i.v. pentylenetetrazol (Lowson et al., 1990) and i.v. lidocaine (Lee et al., 1998). A single dose of 50 mg/kg propofol inhibits self-sustaining SE induced by perforant path stimulation in rats (Holtkamp et al., 2001). Propofol also has been found effective in humans as SE refractory to treatment with i.v. diazepam was controlled by propofol (Kuisma and Roine, 1995). Propofol is comparable in efficacy to treatment with either high dose barbiturates (Stecker et al., 1998) or midazolam (Prasad et al., 2001) in human SE.

Numerous sedative hypnotic agents with agonist activity at the $GABA_A$ receptor ionophore complex have been used to arrest SE and lower the associated mortality rate in rats (Goodman, 1998). Although a $GABA_A$ agonist, propofol also acts to reduce NMDA-mediated whole cell currents (Orser et al., 1995) and is anticonvulsant in NMDA-induced seizures (Ahmad and Pleury, 1995). Propofol is also an antioxidant (Murphy et al., 1992; 1993; Tsuchiya et al., 2001) that may act as a neuroprotectant (Young et al., 1997; Gelb et al., 2002; Wang et al., 2002) by scavenging reactive oxygen species produced during SE. Because of these multiple properties propofol was deemed a viable candidate to test as a possible treatment for Li-pilo-induced SE and by extension as a treatment for organophosphorus nerve agent exposure.

METHODS

Animals: Male, Sprague-Dawley rats obtained from Harlan (Indianapolis, IN) and weighing 290-325 grams at the time of seizure test were used for these experiments. The animals were maintained in a climate-controlled vivarium at 21°C on a 12-hr light/dark cycle and allowed free access to food and water. All animal care and use conformed to the policies of the University of New Mexico Health Sciences Center.

Intracranial implants: The rats were anesthetized with equithesin (a mixture of chloral hydrate, pentobarbital, magnesium sulfate, ethanol, propylene glycol and water) for the surgical implantation of the electrocorticogram (ECoG) recording electrodes. Stainless steel screws were placed bilaterally in the skull 3 mm lateral to midline and equidistant between bregma and lambda. The screws were attached to connector pins by insulated wire. A third screw assembly was placed over the frontal sinus as a reference electrode. Additional screws were set in the skull to serve as anchors. All connector pins were inserted into a McIntyre connector (Ginder Scientific, Ottawa, ON). Screws, wires and connectors were secured in place with dental acrylic cement and the incision site closed with surgical staples. Postoperative antibiotics (25,000 IU Durapen) and analgesics (0.02 mg/kg buprenorphine) were administered. Animals were allowed 7-10 days recovery before seizure testing.

Seizure induction and EcoG recording: The day prior to the seizure induction the rats were administered s.c. 3 mmol/kg lithium chloride (Sigma, St. Louis, MO) dissolved in normal saline. The lithium administration always preceded the pilocarpine administration by 20-24 hr. The following day the animals were placed in a seizure observation cage and connected to a Grass Model 8 electroencephalograph by way of the implanted McIntyre connector for recording of ECoG. Pilocarpine (Sigma) dissolved in normal saline was administered s.c. in a dose of 25 mg/kg following 10 min of baseline ECoG recording. ECoG activity was recorded continuously throughout the experiment. To prevent lethal aspiration, atropine methylnitrate 10 mg/kg s.c. (Sigma) was administered 10 min prior to pilocarpine in those rats administered propofol immediately following pilocarpine (exposure treatment). SE was defined as the occurrence of continuous high amplitude ECoG spiking (Ormandy et al., 1989).

Propofol administration: Propofol was administered i.p. as the commercially available injectable emulsion Propoflo (Baxter Healthcare, New Providence, NJ). A 55 mg/kg dose of propofol was administered either immediately after pilocarpine administration (exposure treatment), following 5 min of SE or following 3 hr of SE as defined by ECoG activity. An additional group was treated with 55 mg/kg propofol after 3 hr SE followed 2 rh later by 20 mg/kg propofol (extended treatment). Rats treated with lithium followed 20-24 hr by s.c. normal saline injection as the vehicle control for

pilocarpine followed 3 hr later by propofol i.p. served as the no seizure control group (No SE).

The rats appeared anesthetized following propofol administration. When the animals lost the righting relflex they were immediately returned to the home cage which had was placed on a heating pad set to 37 C. The animals were placed in the warn cages and covered with bedding to maintain body temperature.

Histological preparation and digital imaging: Animals were sacrificed 24 hr after pilocarpine administration as that is the period after which maximal SE-induced neuropathology is observed in Li-pilo convulsions using hematoxylin and eosin (H&E) staining techniques (Clifford et al., 1987; Fujikawa et al., 1999). All animals were sacrificed by intraaortic perfusion-fixation while anesthetized with equithesin. The animals were initially perfused with heparinized phosphate buffered saline (PBS) (12.5 IU/ml, Sigma) followed by 10% formalin PBS (VWR Scientific Products). Brains were removed and immersed in 10% formalin for a minimum of 24 hr fixation. Following fixation the brains were paraffin embedded and sectioned into 5 μm sections by a rotary microtome (Microm International). Brain sections were mounted on glass slides and stained with H&E.

Two consecutive sections were taken every 125 μm through the brain tissue 0.8 to 4.8 mm posterior to bregma (Paxinos and Watson, 1986). This specific brain region was chosen for analysis because it contains a preponderance of brain nuclei that exhibit the greatest degree of damage from soman (McDonough et al., 1998) and Li-pilo convulsions (Clifford et al., 1987; Motte et al., 1998; Fujikawa et al., 1999; Peredery et al., 2000). Of each pair of consecutive sections, one was used for independent histopathological analysis and the other section for lesion volume measurement.

Tissue sections used for histological analysis of were examined for the presence of necrosis and malacia using a scale of lesion severity developed for assessing soman toxicity (McDonough et al., 1989). The scale was as follows: 0 = none; 1 = minimal = $\leq$ 5% necrotic or malacic tissue; 2 = mild = 6-15% necrotic or malacic tissue; 3 = moderate =16-40% necrotic or malacic tissue; 4 = severe = >40% necrotic or malacic tissue. All sections were graded by a single observer (JG) in a blinded fashion. The median damage

score from a given region across all tissue sections was used as the necrosis score for statistical tests (McDonough et al., 1998).

Tissue sections evaluated using macroscopic lesion volume analysis were viewed using a stereomicroscope (Olympus BH2-RFCA). Images were acquired with a digital camera (Olympus MLH 020550) using the Olympus MagnaFire Camera Imaging and Control software (Version 1.1) and analyzed using Image-Pro Plus software (Version 4.1). Digital images of identical magnification were taken of 11 comparable, consecutive sections from each rat and used for analysis. For each image, the area of the lesion was electronically defined by hand using the Image-Pro area measurement function and calculated as mm^2. The volume of the macroscopic lesion in each animal was determined as the product of the average area of the defined lesions in the consecutive sections and total distance between the sections.

Data and statistical analysis: Comparisons of propofol-induced termination of SE activity were made by student's t-test. Comparisons of the nonparametric histopathological rating score parameters were made using Mann-Whitney U test. Statistical comparison of lesion volumes was determined by analysis of variance (ANOVA) followed by Newman-Kuels post hoc test when a significant difference ($P<0.05$) was determined by ANOVA. Values of $P<0.05$ were considered significant for all statistical tests.

RESULTS

Propofol inhibition of Li-pilo SE: Pilocarpine administration in animals pretreated 20-24 h earlier with lithium induced SE with an average latency of 40 min (n=22, range 26 to 63 min). The average SE duration as defined by continuous high amplitude ECoG activity was 135 min (n=12, range 109-156 min).

Propofol had a significant impact on survival following 3 hr of Li-pilo-induced convulsions. All rats treated with 55 (n=3) or 65 (n=3) mg/kg propofol i.p. after 3 h of SE survived the 24 hr period following pilocarpine administration (Table 1). In contrast, only 3 of 6 animals survived the 24 hr period when administered 50 mg/kg propofol. Propofol completely inhibited all ECoG spiking activity when administered 3 hr after

onset of SE (Fig. 1). Propofol induced a complete suppression of ECoG spiking at which time the animals appeared anesthetized. Twenty-four h following pilocarpine administration the rats were ambulatory and exhibited interictal ECoG spiking (Fig. 1). Following the preliminary range finding study, all subsequent experiments that tested single doses of propofol used 55 mg/kg.

Propofol administered following 5 min of Li-pilo SE inhibited all ongoing seizure activity. The complete suppression of ECoG spiking required an average of 12.2 min (n=5) which was significantly less than the 20.8 min (t-test, $P<0.05$) required following 3 h SE which also completely suppressed all ECoG spiking (n=5). The rats appeared anesthetized when ECoG spiking was arrested. Propofol administered immediately following pilocarpine (exposure treatment) also induced an anesthetized state and prevented the onset of all seizure activity (n=5).

H&E pathological analysis: The histologic lesions, which were characterized at 24 hr after SE, were similar in distribution to previous reports and differed in the degree of severity of necrosis and malacia (Fig. 2). Necrosis was identified initially within neurons that had darkened nuclei and increased cytoplasmic eosinophilia. More severe lesions contained necrosis within adjacent glial cells, intercellular vacuolation and edema that was manifest as malacia with loss of tissue structure in the most severe lesions. Use of the grading scheme permitted an evaluation of the effectiveness of therapy that was consistent with previous reports. Necrosis scores of the piriform and perirhinal cortex of rats administered propofol either immediately following pilocarpine (exposure) or after 5 min of SE (5 min SE) did not differ significantly from that in animals not administered pilocarpine (No SE) (Fig. 3). The piriform and perirhinal regions in rats with only 5 min SE appeared indistinguishable from those not experiencing SE when viewed under low magnification (Fig. 4).

Li-pilo-induced convulsions of 3 hr duration prior to propofol administration produced a significant degree of necrosis and malacia in the piriform and perirhinal cortex. As shown in Fig. 2, malacia was clearly visible in these critical regions. Necrosis scores of these regions (Fig. 3) were significantly greater than in the No SE, exposure or 5 min SE groups (Mann-Whitney U test, $P<0.05$).

The propofol extended treatment group received 55 mg/kg propofol i.p. after 3 hr SE followed 2 h later by an additional 20 mg/kg propofol i.p. The additional 20 mg/kg dose of propofol induced a striking decrease of necrosis and malacia apparent at both low magnification (Fig. 4) and from the necrosis scores derived from histologic examination at higher magnification (Fig. 3). The necrosis scores in the perirhinal cortex were significantly greater than that in the No SE, exposure and 5 min SE groups but were significantly less (Mann-Whitney U test, $P<0.05$) than that in the 3 hr SE group which received only 55 mg/kg propofol (Fig. 3). The results were similar in the piriform cortex except that the difference between the 3 hr SE group and the propofol extended treatment group did not reach statistical significance ($P=0.075$) in the Mann-Whitney U test.

Lesion volume analysis: Extended propofol treatment offered significant protection from malacia in the piriform and perirhinal cortex as determined by macroscopic lesion volume. The No SE, exposure and 5 min SE groups had no detectable macroscopic lesions (Figs. 4 and 6). The 3 hr SE group experienced an average lesion volume of 6.8 mm^3 which was significantly greater (ANOVA and Newman-Kuels post hoc, $P<0.05$) than the average 0.7 mm^3 of the propofol extended treatment group (Fig. 6). Three rats in the propofol extended treatment group had no evidence of a macroscopic lesion in either the piriform or perirhinal cortex. The macroscopic lesion volume of the extended treatment group did not differ significantly from the No SE, exposure or 5 min SE groups.

DISCUSSION

The most significant finding of this study was the neuroprotection induced by propofol. Rats treated with a single 55 mg/kg dose of propofol following 3 hr SE exhibited a significant degree of neuropathology in the piriform and perirhinal cortices as reported previously (Honchar et al., 1983; Clifford et al., 1987; Motte et al., 1998; Peredery et al., 2000; Dube et al., 2000; Dube et al., 2001). Similarly treated rats administered an additional 20 mg/kg propofol 2 h following the initial 55 mg/kg dose exhibited a significant degree of neuroprotection within the perirhinal and piriform cortices. This effect may be due in part to anticonvulsant activity as reported for other

$GABA_A$ receptor agonists that reduce ongoing SE and enhance survival (Lemos and Cavalheiro, 1995; Motte et al., 1998; Covolan and Mello, 2000; Fujikawa et al., 2000; Peredery et al., 2000; Andre et al., 2001; Glien et al., 2001). However, propofol has additional notable pharmacological properties as an NMDA antagonist (Orser et al., 1995; Ahmad and Pleuvry, 1995) and as an antioxidant (Murphy et al., 1992; 1993; Tsuchiya et al., 2001). This is a critical difference from other $GABA_A$ agonists given the predominate role of glutamate and NMDA receptors in both Li-pilo and organophosphorus nerve agent mediated SE (Ormandy et al., 1989; Walton and Treiman, 1991; McDonough and Shih, 1993; McDonough and Shih, 1997). It is hypothesized that the SE-induced NMDA receptor activation leads to an increased production of reactive oxygen species (Lafon-Cazal et al., 1993; Coyle and Puttfarcken, 1993; Michaelis, 1998) that mediate the SE neuropathology (Bruce and Baudry, 1995; Rong and Baudry, 1996; Rong et al., 1999; Peterson et al., 2002). We propose that propofol's NMDA antagonism would attenuate the NMDA receptor–induced excitotoxicity while the antioxidant activity suppresses neuronal destruction by the reactive oxygen species. In support of this hypothesis, propofol has been shown to be anticonvulsant against NMDA-induced convulsions in mice (Ahmad and Pleuvry, 1995) and to be neuroprotective in ischemic stroke by a proposed antioxidant mechanism (Young et al., 1997; Gelb et al., 2002; Wang et al., 2002).

Accumulating evidence supports a role for reactive oxygen species in the cholinergic SE-induced neuropathology of the piriform and perirhinal cortices. Pazdernik et al. (2001) reported a depletion of the endogenous antioxidant glutathione in the piriform cortex the first 24 hr following soman-induced SE, suggesting an oxidative stress phase of the seizures. This is supported by an increased reactive oxygen species production in the piriform cortex during Li-pilo-induced SE as measured by dihydroethidium (Peterson et al., 2002). Additional evidence is provided by a study of HU-211, an NMDA antagonist and antioxidant that induces significant neuroprotection in a model of ischemic stroke (Eshhar et al., 1995). HU-211 also induces significant neuroprotection in soman-induced seizures, reducing the macroscopic lesion volume in rat piriform and perirhinal cortices by 81-86% without affecting the ongoing seizure activity (Filbert et al., 1999). In the present study, propofol extended treatment reduced

the macroscopic lesion volume approximately 89% in addition to terminating the ongoing SE. Propofol may represent a significant advancement in controlling both the SE and neuropathology of cholinergic convulsions.

The present study confirms and extends the previous understanding of Li-pilo convulsions. The failure to observe significant neuropathology following 5 min of Li-pilo-induced SE corroborates previous reports that 30-60 min of pilocarpine or Li-pilo SE is required to observe neuronal damage (Fujikawa, 1996; Lemos and Cavalheiro, 1995; Motte et al., 1998). In addition, propofol terminated ongoing SE in significantly less time (12.2 min) following 5 min of SE than following 3 hr of ongoing convulsions (20.8 min). This supports previous assertions that longer lasting Li-pilo convulsions are more difficult to treat (Jope et al., 1986; Morrisett et al., 1987; Walton and Treiman, 1988; Jones et al., 2002). $GABA_A$ agonists lose potency as Li-pilo SE progresses (Walton and Treiman, 1988; Jones et al., 2002) yet propofol inhibited all motor and electrographic activity after 3 hr SE. Although this may have been a result of $GABA_A$ activity, it is possible that propofol's NMDA antagonist activity (Orser et al., 1995; Ahmed and Pleuvery, 1995) contributed to the anticonvulsant effect. In support of this hypothesis, the NMDA antagonist MK-801 is effective in reducing or terminating Li-pilo SE during the late, glutamatergic phase (Ormandy et al., 1989; Walton and Treiman, 1991).

Severe neuropathology was observed in the piriform and perirhinal cortices following 3 hr SE as previously reported for Li-pilo convulsions (Honchar et al., 1983; Clifford et al., 1987; Motte et al., 1998; Peredery et al., 2000; Dube et al., 2000; Dube et al., 2001). Necrosis scores of H&E sections in the current study are comparable to those reported for soman-induced SE in rats (McDonough et al., 1998). Although the histopathology grading scheme scores the tissue for lesion severity, it does not take into account lesion volume. The macroscopic lesions observed in this study were the result of coalescing malacia the total volume of which was clearly affected by propofol. The lesion volume analysis were used to quantify the extent of the macroscopic malacic lesions. Whether the observed neuroprotection translates to functional neuroprotection or protection from the development of spontaneous limbic seizures requires further study (Lemos and Cavalheiro, 1995; Dube et al., 2000; Dube et al., 2001; Glien et al., 2001).

Propofol possess a spectrum of activity not observed in other treatments of Li-pilo-induced SE. Administered at the time of pilocarpine exposure propofol inhibits seizure onset. Electrographic and behavioral SE of 3 hr duration is completely inhibited by a single 55 mg/kg i.p. dose. When propofol treatment is extended by an additional 20 mg/kg i.p. dose 2 hr after the initial treatment, a significant neuroprotectant effect is induced. These properties would appear to represent a significant improvement over treatments currently employed to control cholinergic convulsions.

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References

Ahmad I., Pleuvry, B.J. (1995) Interactions between opioid drugs and propofol in laboratory models of seizures. Br. J. Anaesth. 74:311-314.

Andre, V., Marescaux, C., Nehlig, A., Fritschy, J.M. (2001) Alterations of hippocampal GABAergic system contribute to development of spontaneous recurrent seizures in the rat lithium-pilocarpine model of temporal lobe epilepsy. Hippocampus 11, 452-468.

Bruce, A.J., Baudry, M. (1995) Oxygen free radicals in rat limbic structures after kainite-induced seizures. Free Radic. Biol. Med. 18(6), 993-1002.

Clifford, D.B., Olney, W., Maniotis, A., Collins, R.C., Zorumski, C.F. (1987) The functional anatomy and pathology of lithium-pilocarpine and high-dose pilocarpine seizures. Neuroscience 23(3), 953-968.

Coyle, S.T., Puttfarcken, P. (1993) Oxidative stress, glutamate and neurodegenerative disorders. Science 262, 689-694.

Covolan, L., Mello, L.E.A.M. (2000) Temporal profile of neuronal injury following pilocarpine or kainic acid-induced status epilepticus. Epilepsy Res. 39, 133-152.

Dubé, C., Boyet, S., Marescaux, C., Nehlig, A. (2000) Progressive metabolic changes underlying the chronic reorganization of brain circuits during the silent phase of the lithium-pilocarpine model of epilepsy in the immature and adult rat. Exp. Neurol. 162, 146-157.

Dubé, C., Boyet, S., Marescaux, C., Nehlig, A. (2001) Relationship between neuronal loss and interictal glucose metabolism during the chronic phase of the lithium-pilocarpine model of epilepsy in the immature and adult rat. Exp. Neurology 167, 227-241.

Eshhar, N., Striem, S., Kohen, R., Tirosh, O., Biegon, A. (1995) Neuroprotective and antioxidant activities of HU-211 a novel NMDA receptor antagonist. Eur. J. Pharmacol. 283:19-29.

Filbert, M.G., Forster, J.S., Smith, C.D., Ballough, G.P.H. (1999) Neuroprotective effects of HU-211 on brain damage resulting from soman-induced seizures. Ann. NY Acad. Sci. 890:505-514.

Fujikawa, D.G. (1996) The temporal evolution of neuronal damage from pilocarpine-induced status epilepticus. Brain Res. 725:11-22.

Fujikawa, D.G., Shinmei, S.S., Cai, B. (1999) Lithium-pilocarpine-induced status epilepticus produces necrotic neurons with internucleosomal DNA fragmentation in adult rats. Eur. J. Neurosci. 11, 1605-1614.

Fujikawa, D.G., Shinmei, S.S., Cai, B. (2000) Seizure-induced neuronal necrosis: implication for programmed cell death mechanisms. Epilepsia 41(S6), S9-S13.

Gelb, A.W., Bayona, N.A., Wilson, J.X., Cechetto, D.F. (2002) Propofol anesthesia compared to awake reduces infarct size in rats. Anesthesiology 96: 1183-1190.

Glien, M., Brandt, C., Potschka, H., Voigt, H., Ebert, U., Loscher, W. (2001) Repeated low-dose treatment of rats with pilocarpine: low mortality but high proportion of rats developing epilepsy. Epilepsy Res. 46:111-119.

Goodman, J.H. (1998) Experimental models of status epilepticus. In: Peterson, S.L., Albertson, T.E. (Eds), Neuropharmacology Methods in Epilepsy Research. CRC Press, New York, pp 95-125.

Holtkamp, M., Tong, X., Walker, M.C. (2001) Propofol in subanesthetic doses terminates status epilepticus in a rodent model. Ann. Neurol. 49, 260-263.

Honchar, M.P., Olney, J.W., Sherman, W.R. (1983) Systemic cholinergic agents induce seizures and brain damage in lithium-treated rats. Science 220, 323-334.

Jones, D., Esmaeil, N., Maren, S., Macdonald, R. (2002) Characterization of pharmacoresistance to benzodiazepines in the rat Li-pilocarpine model of status epilepticus. Epilepsy Res. 50(3): 301-312.

Jope, R.S., Morrisett, R.A., Snead, O.C. (1986) Characterization of lithium potentiation of pilocarpine-induced status epilepticus in rats. Exp. Neurol. 91, 471-480.

Kuisma, M., Roine, R.O. (1995) Propofol in prehospital treatment of convulsive status epilepticus. Epilepsia, 36(12), 1241-1243.

Lafon-Cazal, M., Pietri, S., Culcasi, M., Bockaert, J. (1993) NMDA-dependent superoxide production and neurotoxicity. Nature 364, 535-537.

Lee, V.C., Moscicki, J.C., DiFazio, C.A. (1998) Propofol sedation produces dose-dependent suppression of lidocaine-induced seizures in rats. Anesth. Analg. 86, 652-657.

Lemos, T., Cavalheiro, E.A. (1995) Suppression of pilocarpine-induced status epilepticus and the late development of epilepsy in rats. Brain Res. 102, 423-428.

Lowson, S., Gent, J.P., Goodchild, C.S. (1990) Anticonvulsant properties of propofol and thiopentone: comparison using two tests in laboratory mice. Br. J. Anaesth. 64, 59-63.

McDonough, J.H., Shih, T.M. (1993) Pharmacological modulation of soman-induced seizures. Neurosci. Biohehav. Rev. 17, 203-215.

McDonough, J.H., Jr, Shih, T.M. (1997) Neuropharmacological mechanisms of nerve agent-induced seizure and neuropathology. Neurosci. Biobehav. Rev. 21(5), 559-579.

McDonough, J.H., Clark, T.R., Slone, T.W., Zoeffel, D., Brown, K., Kim, S., Smith, C.D. (1998) Neural lesions in the rat and their relationship to EEG delta activity following seizures induced by the nerve agent soman. Neurotoxicology 19(3), 381-392.

McLean, M.J., Gupta, R.C., Dettbarn, W.D., Wamil, A.W. (1992) Prophylactic and therapeutic efficacy of memantine against seizures produced by soman in the rat. Toxicol. Appl. Pharmacol. 112, 95-103.

Michaelis, E.K. (1998) Molecular biology of glutamate receptors in the central nervous system and their role in excitotoxicity, oxidative stress and aging. Prog. Neurobiol. 54, 369-416.

Morrisett, R.A., Jope, R.S., Snead, O.C. (1987) Effects of drugs on the initiation and maintenance of status epilepticus induced by administration of pilocarpine to lithium-pretreated rats. Exp. Neurol. 97, 193-200.

Motte, J., da Silva Fernandes, M.J., Baram, T.Z., Nehlig, A. (1998) Spatial and temporal evolution of neuronal activation, stress and injury in lithium-pilocarpine seizures in adult rats. Brain Res. 798, 61-72.

Murphy, P.G., Myers, D.S., Davies, M.J., Webster, N.R., Jones, J.G. (1992) The antioxidant potential of propofol (2,6-diisopropylphenol). Br. J. Anaesth. 68(6). 613-618.

Murphy, P.G., Bennett, J.R., Myers, D.S., Bavies, M.J., Jones, J.G. (1993) The effect of propofol anesthesia on free radical-induced lipid-peroxidation in rat-liver microsomes. Eur. J. Anaesth. 10(4), 261-266.

Ormandy, G.C., Jope, R.S., Snead, O.C. (1989) Anticonvulsant actions of MK-801 on the lithium-pilocarpine model of status epilepticus in rats. Exp. Neurol. 106, 172-180.

Orser, B.A., Bertlik, M., Wang, L.Y., MacDonald, J.F. (1995) Inhibition of propofol (2,6 di-isopropylphenol) of the N-methyl-D-asparate subtype of glutamate receptor in cultured hippocampal neurons. Br. J. Pharmacol. 116(2), 1761-1768.

Paxinos, G., Watson, C. (1986) The rat brain in stereotaxic coordinates. Orlando, FL: Academic Press. P 1-126.

Pazdernik, T.L., Emerson, M.R., Cross, R., Nelson, S.R., Samson, F.E. (2001) Soman-induced seizures: limbic activity, oxidative stress and neuroprotective proteins. J. Appl. Toxicol. 21, S87-S94.

Peredery, O., Persinger, M.A., Parker, G., Mastrosov, L. (2000) Temporal changes in neuronal dropout following inductions of lithium/pilocarpine seizures in the rat. Brain Res. 881, 9-17.

Peterson, S.L., Morrow, D., Liu, S., Liu, K.J. (2002) Hydroethidine detection of superoxide production during the lithium-pilocarpine model of status epilepticus. Epilepsy Res. 49, 226-238.

Prasad, A., Worrall, B.B., Bertam, E.H., Bleck, T.P. (2001) Propofol and midazolam in the treatment of refractory status epilepticus. Epilepsia 42(3), 380-386.

Rasmussen, P.A., Yang, Y., Rutecki, P.A. (1996) Propofol inhibits epileptiform activity in rat hippocampal slices. Epilepsy Res. 25(3), 169-175.

Rong, Y., Baudry, M. (1996) Seizure activity results in a rapid induction of nuclear factor-κB in adult but not juvenile rat limbic structures. J. Neurochem. 67(2), 662-668.

Rong, Y., Doctrow, S.R., Tocco, G., Baudry, M. (1999) EUK-134, a synthetic superoxide dismutase and catalase mimetic, prevents oxidative stress and attenuates kainite-induced neuropathology. Proc. Natl. Acad. Sci. 96, 9897-9902.

Shih, T.M. (1990) Anticonvulsant effects of diazepam and MK-801 in soman poisoning. Epilepsy Res. 7, 105-116.

Shih, T.M., McDonough, J.H., Koplovitz, I. (1997) Evaluation of anticonvulsant drugs for soman-induced seizure activity. J. Amer. Col. Toxicol. 15(Suppl. 2), S43-S60.

Solberg, Y., Belkin, M. (1997) The role of excitotoxicity in organophosphorous nerve agents central poisoning. TIPS 18(6), 183-185.

Stecker, M.M., Kramer, T.H., Raps, E.C., O'Meeghan, R.O., Dulaney, E., Skaar, D.J. (1998) Treatment of refractory status epilepticus with propofol: clinical and pharmacokinetic findings. Epilepsia 39(1), 18-26.

Tsuchiya, M., Asada, A., Maeda, K., Ueda, Y., Sato, E.F., Shindo, M, Inoue, M. (2001) Propofol versus midazolam regarding their antioxidant activities. Am. J. Respir. Crit. Care Med. 163: 26-31.

Walton, N.Y., Treiman, D.M. (1988) Response to status epilepticus induced by lithium and pilocarpine to treatment with diazepam. Exp. Neurol. 101:267-275.

Walton, N.Y., Treiman, D.M. (1991) Motor and electroencephalographic response of refractory experimental status epilepticus in rats to treatment with MK-801, diazepam, or MK-801 plus diazepam. Brain Res. 553, 97-104.

Wang, J., Yang, X., Camporesi, C., Yang, Z., Bosco, G., Chen, C., Camporesi, E. (2002) Propofol reduces infarct size and striatal dopamine accumulation following transient middle cerebral artery occlusion: a microdialysis study. Eur. J. Pharmacol. 452(3): 303-308.

Young, Y., Menon, D.K., Tisavipat, N., Matta, B.F., Jones, J.G. (1997) Propofol neuroprotection in a rat model of ischaemia reperfusion injury. Eur. J. Anaesth. 14(3), 320-326.

Table 1. Propofol induced a dose-dependent increase in 24 h survival following Li-pilo SE. Propofol was administered i.p. 3 h after the onset of SE as defined by continuous high amplitude ECoG spiking.

Propofol (mg/kg)	N	Percent Survival
50	6	50%
55	3	100%
65	4	100%

Figure Legends

Figure 1. Representative ECoG tracings from a rat in Li-pilo-induced SE that was treated with 55 mg/kg propofol. Propofol was administered 3 h after the onset of SE as defined by continuous high amplitude ECoG spiking. An example of ECoG activity representative of SE is shown in the tracing labeled "30 min SE". Propofol suppressed all ECoG spiking as shown in the tracing labeled "30 min after propofol". Rats appeared anesthetized when the ECoG spiking was suppressed. Interictal spiking was present 24 h following pilocarpine administration as seen in the bottom tracing.

Figure 2. Representative examples of lesion grades and normal control brain. All photographs were taken at 120X original magnification of H&E stained brain. A: piriform cortex, minimal lesion (score = 1). Scale bar represents 100 μm. There are a few dark necrotic neuron nuclei with over 95% of the cells normal. B: piriform cortex, mild lesion (score = 2). Between 6-15% of the neuron nuclei are dark and shrunken and the cytoplasms are more distinct due to increased eosinophilia indicating necrosis. There is mild vacuolation. C: perirhinal cortex, moderate lesion (score = 3). Between 16-40% of the neurons are necrotic, which includes pyknosis, karyorrhexis, and loss of

cytoplasmic detail. There is moderate vacuolation, some of which are coalescing. D: piriform cortex, severe lesion (score = 4). Greater than 40% of the neurons are necrotic. The nuclei are dark, shrunken and pyknotic. Affected cytoplasms have increased eosinophilia. There is marked coalescing vacuolation and malacia.

Figure 3. Comparison of neuronal damage in piriform and perirhinal cortex as determined by pathological ratings in H&E sections. Using necrosis scores the neuropathology in the 3 hr SE and propofol extended treatment (Ext Txmt) groups was significantly greater than that of all other groups (1=significant difference from No SE, Exposure, 5 min SE groups, Mann Whitney U-test, $P<0.05$). In perirhinal cortex the neuropathology was significantly less in the propofol extended treatment group than the 3 hr SE group (2=significant difference from 3 hr SE, Mann-Whitney U-test, $P<0.05$). Although similar, the difference between the 3 hr SE groups and propofol extended treatment groups in the piriform cortex did not reach statistical significance (Mann-Whitney U test, $P=0.075$).

Figure 4. Representative low magnification H&E sections of rat brains demonstrating the extent of Li-pilo-induced neuropathology and the neuroprotection induced by propofol. No lesion is visible in the brain of the rat that experienced 5 min of SE prior to propofol (5 minutes SE), the brain is indistinguishable from the control brain (No SE). An extensive neuronal lesion is clearly visible in the piriform and perirhinal cortex of the rat that experienced 3 hr SE prior to 55 mg/kg propofol administration (3 hours SE). The rat in the extended treatment received 55 mg/kg propofol after 3 hr SE followed 2 hr later by 20 mg/kg propofol. With the additional 20 mg/kg dose being the only difference, the extended treatment rat exhibited a marked decrease in piriform and perirhinal neuropathology. PRh: perirhinal cortex, Pir: piriform cortex, BLA: basolateral amygdala. Scale bar equals 1 mm.

Figure 5. Comparison of neuronal damage in piriform and perirhinal cortex as determined by macroscopic lesion volume. When perirhinal macroscopic lesions occurred they were continuous with those in the piriform cortex. Therefore, lesions were

considered in their entirety and not separated by region. The macroscopic lesion volume was significantly greater in the 3 hr SE group as compared to all others (1=significantly different from No SE, exposure (Exp), 5 min SE and extended treatment (Ext Txmt) groups, ANOVA and Newman-Kuels post hoc, $P<0.05$).

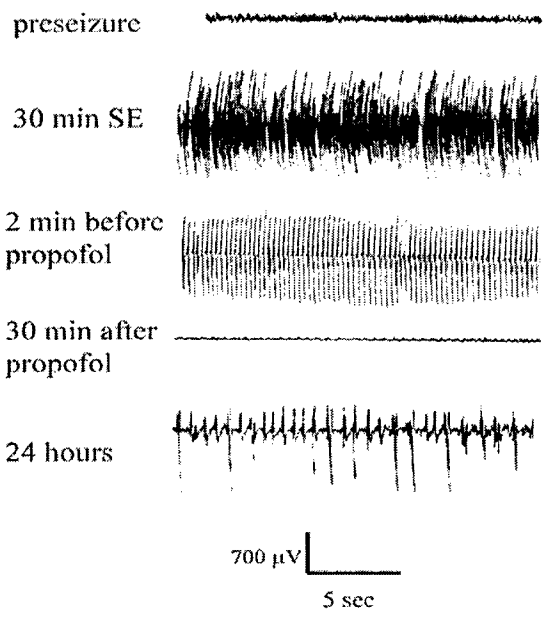
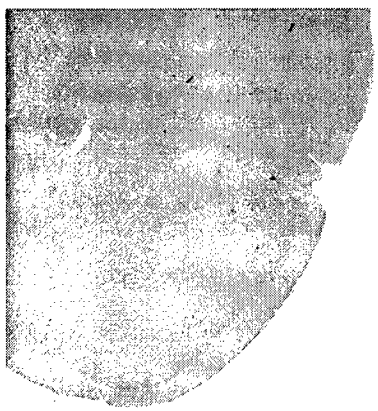

preseizure
30 min SE
2 min before propofol
30 min after propofol
24 hours
700 μV
5 sec

Fig. 1



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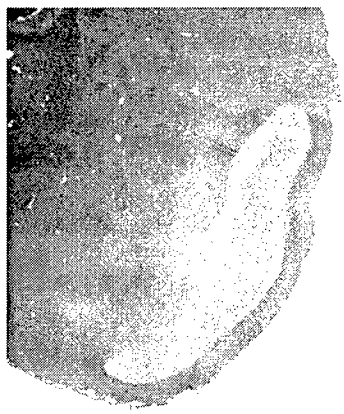
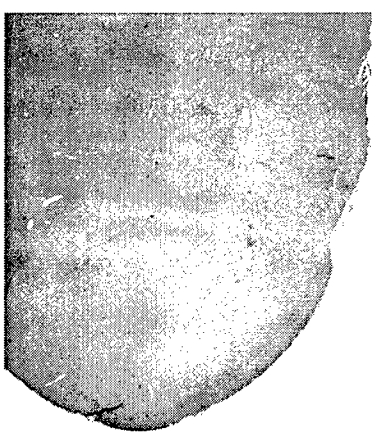


3 hours



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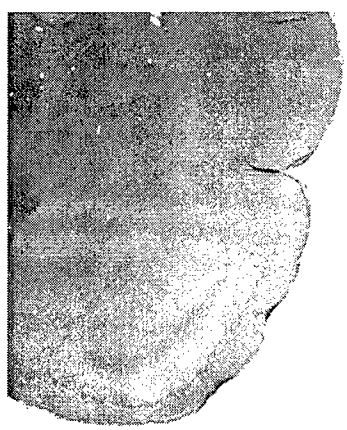


Extended

Fig. 4

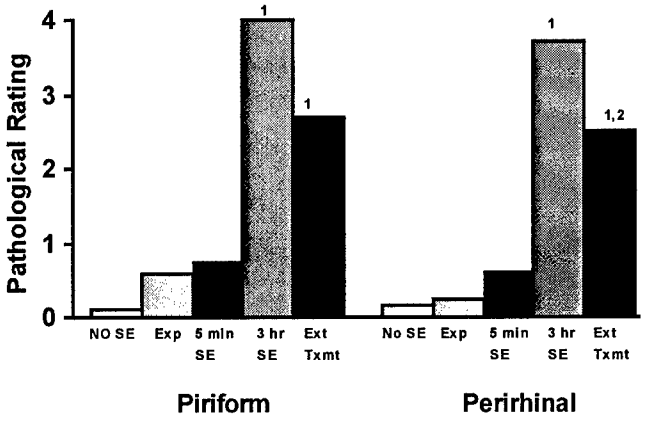


Fig. 3

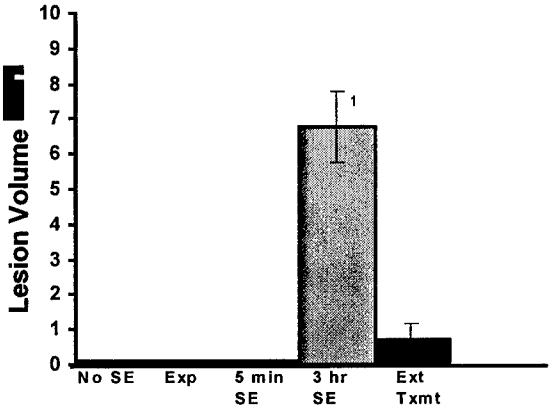
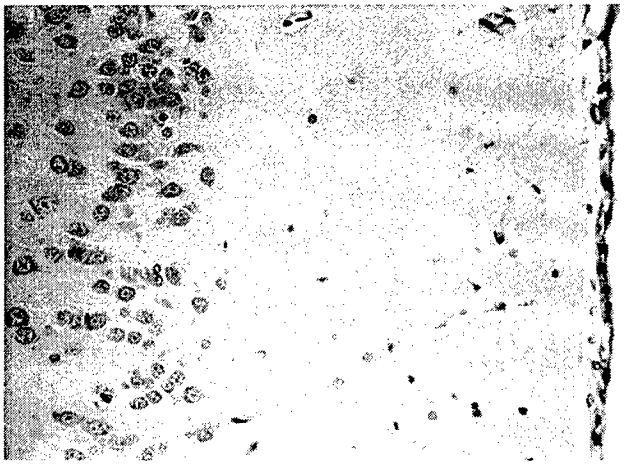


Fig. 5



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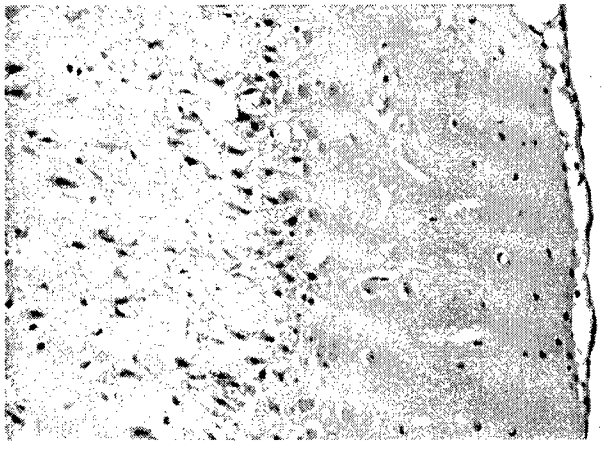

3 hours SE